

Study of the Antibacterial Activity of *Artemisia absinthium* Against *E. coli* Bacteria Isolated from Urinary Tract Infections

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Abstract

Twenty clinically diagnosed samples were collected using transport medium swabs from the operating room at Al-Amal Specialized Laboratory in Iraq between November 2025 and February 10, 2026. Antibiotic susceptibility testing was performed on the 20 *Escherichia coli* samples. The results showed a clear difference in the bacterial isolates' response to the tested antibiotics. Imipenem demonstrated the highest inhibition rate, reaching 24.55% (100%), compared to the other antibiotics used in the study. This indicates the importance of this antibiotic in treating bacterial infections caused by these isolates. The results also showed that the antibiotic gentamicin exhibited good activity against the tested bacterial isolates, reaching 18.85 (90%), while moxifloxacin showed moderate activity (13.55), with varying responses among the isolates. Tetracycline showed limited activity (2.70, 80%), while all isolates were resistant to BY, which may reflect the prevalence of bacterial resistance mechanisms to this antibiotic due to its widespread use in treatment. The results presented in this study indicate that the hot water extract of *Artemisia absinthium* exhibited antibacterial activity that increased with increasing concentration. The 100% concentration recorded the highest inhibition zone diameter of 20 mm, indicating high efficacy in inhibiting bacterial growth. The 50% concentration recorded an inhibition zone diameter of 17 mm, indicating moderate antibacterial activity. The 25% concentration showed the least inhibitory activity with a diameter of 13 mm.

Keywords: *Artemisia absinthium*, *E-Coli*, bacteria, inhibition, moxifloxacin

INTRODUCTION

Escherichia coli is a member of the Enterobacteriaceae family. It is a Gram-negative, rod-shaped bacterium that can be motile or non-motile, aerobic or anaerobic, facultatively anaerobic, and lactose-fermenting. Most strains also ferment rhamnose and sorbitol. They produce the enzyme β -glucuronidase, and their optimal growth temperature is 36–37°C (Wanger et al., 2017; Jawetz et al., 2016). They produce oxidase and are negative for catalase oxidase, positive for catalase, and produce indole. They do not consume citrate, are positive for methyl red, and negative for Vogase-Proskauer (Hemraj et al., 2013) [13].

It naturally inhabits the intestines of humans and animals and is also an opportunistic bacterium. Opportunistic pathogens cause many diseases such as diarrhea, meningitis, sepsis, and bacteremia. They are among the most common bacteria causing urinary tract infections, accounting for approximately 90% of urinary tract infections worldwide, and are most prevalent in childhood (Hadi et al., 2014; Shweikh and Jassim, 2016) [1, 2].

The pathogenicity of these bacteria is attributed to their possession of several virulent factors. These factors include iron chelates, siderophores, cytotoxic

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necrotizing factors (CNF), and surface structures such as flagella, capsules, and lipopolysaccharides (LPS). These components give the bacteria antigenic properties by producing the flagellar antigen H and the somatic antigen O (capsular flagellum). The bacteria (antigen K) also possess cilia (fimbriae or pili) that help them adhere to host tissues, thus enabling them to form biofilms (Terlizzi et al., 2017; Zowawi et al., 2015) [25, 31].

Escherichia coli is characterized by multidrug resistance (MDR) (Laird, 2016). This high resistance to antibiotics is due to the presence of resistance enzymes, such as β -lactamases, which confer resistance to β -lactams, and enzymes that confer resistance to aminoglycosides and quinolones. These bacteria also possess other mechanisms that confer antibiotic resistance, such as altering cell membrane permeability, changing the target site, inhibiting protein synthesis, and possessing efflux pumps. These mechanisms confer resistance to antibiotics such as macrolides, rifampin, and novobiocins (Kapoor et al., 2017) [3, 4].

Artemisia absinthium is characterized by its content of numerous active compounds, volatile oils, and alkaloids (Rizk, 1986). It also contains artemisinin, the plant's primary component, as well as sesquiterpene lactones, which are largely responsible for the importance of this plant in medicine and pharmacy. Plants of the genus *Artemisia* have been used in traditional medicine since ancient times. These species have been used as an analgesic, antibacterial, antispasmodic, and anticoagulant (Abou El-Hamd et al., 2010). In some Middle Eastern countries, *Artemisia absinthium* is used as an antidiabetic (Iriadam, 2006), as well as a stimulant, tonic, and heart tonic, and as an emmenagogue [5].

Given the medical importance of *E. coli* bacteria and their association with urinary tract infections and antibiotic resistance, this study was conducted according to the following objectives:

- Conducting sensitivity tests for different groups of antibiotics.
- Obtaining a hot aqueous extract of *Artemisia absinthium*.
- Testing the sensitivity of isolates to different concentrations of the hot aqueous extract of *Artemisia absinthium*.
- Phenotypically investigating the ability of the hot aqueous extract of *Artemisia absinthium* to inhibit the growth of *E. coli* bacteria and comparing the results with the sensitivity test [6, 7].

METHODS

Twenty Clinical samples were collected using transport medium swabs from the operating room at Al-Amal Specialized Laboratory in Iraq between November 2025 and February 10, 2026.

Prior to sample collection, the operating room was sterilized according to hospital protocols, including the floors, walls, operating tables, lighting units, and anesthesia equipment (Berghaus et al., 2018; Carroll et al., 2019).

The samples were stored under 2–8°C refrigerated conditions to ensure the preservation of microbial viability.

Preparation of Culture Media

Dried culture media were dissolved in distilled water according to the manufacturer's instructions and then autoclaved at 121°C for 15 minutes. The medium was then allowed to cool to 45–50°C before being transferred to sterile Petri dishes.

Initial Isolation Swabs were cultured on Blood agar and MacConkey agar using the quadrant method to obtain separate colonies (MacFaddin, 2000).

The plates were incubated at 37°C for 24 hours under aerobic conditions, with slow-growing colonies monitored for up to 48 hours if needed (Cheesbrough, 2010).

Colonies were examined for morphology, size, color, and lytic activity on Blood agar, and for lactose fermentation characteristics on MacConkey agar. Colony counts were recorded in CFU/surface area. Representative colonies were transferred to fresh culture media to obtain pure isolates and stored at 4°C [8, 9].

Antibiotic Susceptibility Testing

The susceptibility of isolates to antibiotics was assessed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar.

Bacterial inoculum was prepared from freshly grown colonies, and the bacterial suspension density was adjusted to a 0.5 McFarland standard. The suspension was distributed on the medium, and the following antibiotic disks were added: Gentamicin (GM), Imipenem (IPM), Moxifloxacin (MXF), Polymyxin B (PY), and Tetracycline (T).

After incubation at 37°C for 18–24 hours, the diameters of the inhibition zones were measured in millimeters to determine susceptibility [10].

Preparation of the Hot Aqueous Extract of *Artemisia absinthium*

The extract was prepared according to Al-Mansour's method (1995) as follows:

- *Artemisia absinthium* leaves were ground, and 50 grams of dry powder were obtained.
- The powder was mixed with 500 mL of 70% ethanol in a 500 mL beaker.
- The mixture was left at room temperature for 24 hours with the beaker tightly covered to prevent contamination.
- The mixture was filtered through cheesecloth to remove suspended solids.
- The filtrate was placed in a heat dryer at 40°C for 24 hours to yield 10 grams of new powder.
- This powder was dissolved in 100 mL of boiled distilled water to obtain a 100% base concentration. In this study, only the 100% extract was used on the test plates to evaluate its antibacterial activity.

Twenty bacterial samples were distributed so that each sample was tested on one plate containing the 100% extract, in addition to a control plate, to evaluate the antibacterial efficacy at full concentration compared to the control [11].

Use of the Extract on Samples

Sensitivity Testing Against Hot Water Artemisia Extract: The sensitivity of the studied isolates to three dilutions (100%, 50%, and 25%) of the hot water extract of *Artemisia* was assessed using the burrow diffusion method on Mueller-Histone agar. Bacterial inoculum was prepared from colonies to test for growth and inhibition of oral suspension density according to the Heckler 0.5 standard. The suspension was then distributed onto the samples prepared with the hot extract. After incubation at 37°C for 24 hours, the diameters of the salt inhibition zones were measured to determine the sensitivity of the bacterial cultures to the extract.

Statistical Analysis

Data was analyzed using IBM SPSS Statistics v26. Descriptive statistics included mean, standard deviation, and frequencies. Comparisons between samples were performed using one-way ANOVA (Field, 2018; Zar, 2010). The probability value $p \leq 0.05$ was adopted to determine statistical significance. This approach ensures an accurate interpretation of the results and a reliable assessment of the antibacterial efficacy of the extract at full concentration compared to the control [12, 13].

RESULTS AND DISCUSSION

This study presents the results obtained from antibiotic susceptibility testing of bacterial isolates, as well as an evaluation of the antibacterial activity of *Artemisia absinthium* extract. In this study, twenty *E. coli* bacterial isolates from urinary tract infections were tested. Their antibacterial activity was evaluated using two main methods:

Antibiotic susceptibility testing using the disk diffusion method. Five antibiotics were tested: gentamicin (GM), imipenem (IPM), moxifloxacin (MXF), polymyxin B (PY), and tetracycline (T), as shown in Table 1 and Figure 1.

Table 1. Diameters of the inhibition zone (min) of antibiotics against 20 bacterial isolates.

| Bacteria isolates | HLG | IPM | MXF | PY | T |
|-------------------|-----|-----|-----|----|----|
| 1 | 17 | 22 | 0 | 0 | 17 |
| 2 | 19 | 24 | 23 | 0 | 19 |
| 3 | 18 | 22 | 25 | 0 | 18 |
| 4 | 17 | 17 | 0 | 0 | 0 |
| 5 | 17 | 25 | 28 | 0 | 0 |
| 6 | 18 | 24 | 18 | 0 | 0 |
| 7 | 20 | 29 | 12 | 0 | 0 |
| 8 | 10 | 26 | 18 | 0 | 0 |
| 9 | 21 | 28 | 12 | 0 | 0 |
| 10 | 21 | 27 | 10 | 0 | 0 |
| 11 | 22 | 29 | 13 | 0 | 0 |
| 12 | 20 | 25 | 13 | 0 | 0 |
| 13 | 18 | 23 | 11 | 0 | 0 |
| 14 | 20 | 26 | 10 | 0 | 0 |
| 15 | 19 | 28 | 16 | 0 | 0 |
| 16 | 18 | 23 | 12 | 0 | 0 |
| 17 | 19 | 22 | 12 | 0 | 0 |
| 18 | 21 | 29 | 13 | 0 | 0 |
| 19 | 20 | 27 | 12 | 0 | 0 |
| 20 | 22 | 29 | 13 | 0 | 0 |

Note: HLG: High Level Gentamicin, IMP: Imipenem, MXF: Moxifloxacin, T: Tetracycline.

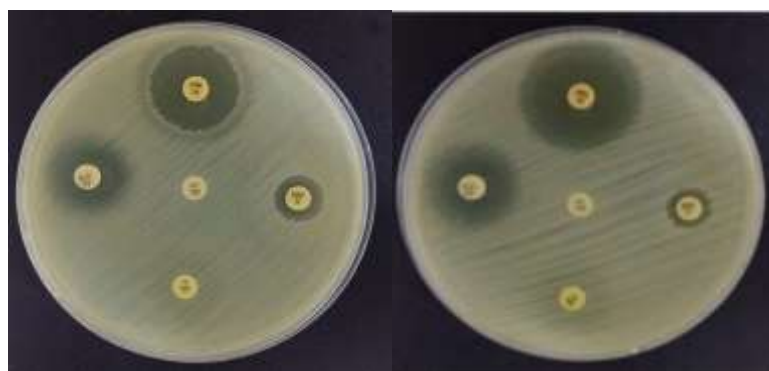


Figure 1. Illustrates the sensitivity of bacteria to antibiotic tablets.

Antibiotic Susceptibility Pattern of Bacterial Isolates

The susceptibility of the 20 bacterial isolates to antibiotics was assessed using the Kirby-Bauer disc diffusion method. Table 2 shows the diameters of the inhibition zones produced by the tested antibiotics. The results indicate a clear difference in the susceptibility of the bacterial isolates to the tested antibiotics, with imipenem exhibiting the highest inhibition zones compared to the other antibiotics. Descriptive Statistical Analysis of Antibiotics

Descriptive statistical analysis was performed to evaluate the antibacterial activity of the tested antibiotics [14].

Table 2. Descriptive statistical analysis of inhibition zone diameters.

| Antibiotic | N | Mean (mm) | Standard deviation | Lowest value | Highest value |
|------------|----|-----------|--------------------|--------------|---------------|
| GM | 20 | 18.85 | 2.62 | 10 | 22 |
| IPM | 20 | 24.55 | 3.27 | 17 | 29 |
| MXF | 20 | 13.55 | 6.83 | 0 | 28 |
| B PY | 20 | 0.00 | 0.00 | 0 | 0 |
| T | 20 | 2.70 | 6.60 | 0 | 19 |

The results show that imipenem had the highest mean inhibition zone, indicating high activity against the bacterial isolates. Gentamicin also showed good antibacterial activity, while moxifloxacin showed moderate activity with some variation among the isolates. Polymyxin B showed no inhibitory activity, while tetracycline showed weak activity.

The results show that imipenem exhibited the highest mean region of inhibition, indicating high efficacy against the bacterial isolates. Gentamicin also demonstrated good antibacterial activity, while moxifloxacin showed moderate efficacy with some variability among the isolates. Polymyxin B showed no inhibitory activity, while tetracycline showed weak activity [15].

Statistical Analysis of Antibiotics

One-way analysis of variance (ANOVA) was used to determine whether the differences between the tested antibiotics were statistically significant. The results showed statistically significant differences ($P < 0.05$) between the tested antibiotics (Table 3).

Table 3. Statistical analysis of antibiotics.

| Antibiotic | Mean \pm standard deviation | P |
|--------------|-------------------------------|--------|
| Gentamicin | 2.62 \pm 18.85 | <0.001 |
| Imipenem | 3.27 \pm 24.55 | <0.001 |
| Moxifloxacin | 6.83 \pm 13.55 | 0.002 |
| Polymyxin B | 0.00 \pm 0.00 | مرجع |
| Tetracycline | 6.60 \pm 2.70 | 0.041 |

Antibacterial Activity of (*Artemisia absinthium*) Extraction

the antibacterial activity of (*Artemisia absinthium*) extract prepared by hot hydro-extraction at concentrations of 25%, 50%, and 100%. was evaluated against 20 bacterial isolates using the agar diffusion method (Figure 1).

The diameters of the inhibition zones were measured in millimeters (mm), and the results were analyzed using descriptive and inferential statistics to determine any significant differences in antibacterial activity. The results shown in Table 4 indicate that *Artemisia absinthium* extract exhibited antibacterial activity that increased with increasing concentration. The 100% concentration recorded the highest inhibition zone diameter of 20 mm, demonstrating high efficacy in inhibiting bacterial growth. The 50% concentration recorded an inhibition zone diameter of 17 mm, indicating moderate antibacterial activity. The 25% concentration showed the lowest inhibitory activity, with a diameter of 13 mm. These results demonstrate a direct relationship between the concentration of the plant extract and its antibacterial activity, with the inhibitory effect on bacterial growth increasing with increasing extract concentration (Figure 2).

Table 4. Effect of (*artemisia absinthium*) extract on bacterial isolates.

| Extract concentration(%) | Diameter of the inhibition zone (m) |
|--------------------------|-------------------------------------|
| 100% | 20 |
| 50% | 17 |
| 25% | 13 |

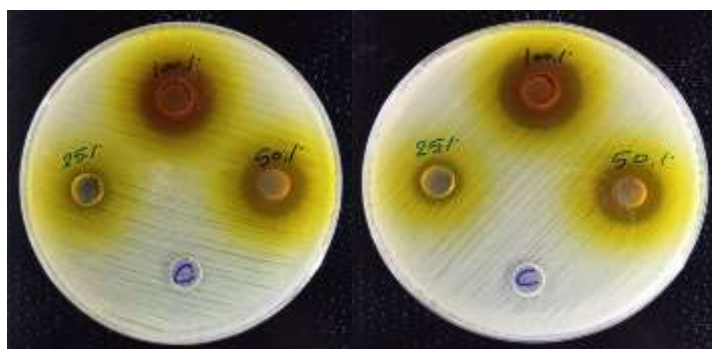


Figure 2. Illustrates the sensitivity of bacteria to (*Artemisiaabsinthiu* extract).

The results of the current study showed a clear difference in the response of bacterial isolates to the tested antibiotics. Imipenem demonstrated the highest activity in inhibiting bacterial growth compared to the other antibiotics used in the study. This result reflects the high ability of carbapenem antibiotics to inhibit bacterial cell wall synthesis. This class of antibiotics works by disrupting peptidoglycan-synthetic enzymes essential for cell wall formation, ultimately leading to bacterial cell death. Several studies have indicated that carbapenems are among the most effective antibiotics against Gram-negative bacteria, especially in cases where resistance to other antibiotics is present (Nordmann et al., 2011; Papp-Wallace et al., 2011) [22, 23].

The results also showed that the antibiotic gentamicin possesses good antibacterial activity, registering a relatively high average number of inhibition zones. This is attributed to its mechanism of action, which involves inhibiting protein synthesis within the bacterial cell by binding to the 30S ribosomal subunit. This disrupts translation and the production of proteins essential for bacterial growth. Numerous studies have shown that gentamicin is an effective antibiotic in treating various bacterial infections caused by Gram-negative bacteria (Krause et al., 2016) [16–18].

In contrast, the antibiotic moxifloxacin demonstrated moderate efficacy against bacterial isolates, with varying responses among different isolates. This is attributed to the differing mechanisms of bacterial resistance to this class of antibiotics. Fluoroquinolones work by inhibiting the enzymes DNA gyrase and topoisomerase IV, which are responsible for bacterial DNA replication. However, genetic mutations in these enzymes can reduce bacterial sensitivity to these antibiotics (Hooper & Jacoby, 2016)

As for the antibiotic tetracycline, the results showed relatively low efficacy in inhibiting the growth of the tested bacterial isolates. This is attributed to the widespread prevalence of bacterial resistance mechanisms to this antibiotic, such as the presence of drug-extraction pumps or ribosomal protection proteins, that prevent the drug from binding to the bacterial ribosome. Several studies have indicated that the widespread and unregulated use of tetracyclines has led to an increase in the emergence of resistant bacterial strains (Chopra & Roberts, 2001).

Antibacterial activity of *Artemisia absinthium* extract

The results of this study showed that *Artemisia absinthium* extract possesses varying degrees of antibacterial activity depending on the concentration used. The 100% concentration resulted in the highest inhibition zone diameter of 20 mm, while the 50% concentration resulted in a diameter of 17 mm, and the 25% concentration resulted in the lowest diameter of 13 mm.

These results indicate a direct relationship between the concentration of the plant extract and its antibacterial activity, with the inhibitory effect on bacterial growth increasing with increasing extract concentration. This effect can be explained by the presence of several active chemical compounds in *Artemisia absinthium*, such as phenolic compounds, flavonoids, and terpenes, which possess antibacterial properties. These compounds disrupt the integrity of the bacterial cell membrane or inhibit certain vital processes within the bacterial cell, leading to inhibited growth or cell death (Bilia et al., 2014).

Furthermore, several studies have indicated that plants of the genus *Artemisia* possess diverse pharmacological properties, including antibacterial, antifungal, and antiviral activity. This is due to the presence of a wide range of bioactive compounds in these plants that can influence the growth of microorganisms (Efferth, 2017).

The results of this study are consistent with previous research showing that *Artemisia absinthium* extracts can inhibit the growth of many types of pathogenic bacteria. These studies demonstrated that the active compounds in the plant can damage the bacterial cell membrane or affect its permeability, leading to leakage of cellular components (Abad et al., 2012).

Comparison between Antibiotics and Plant Extract

When comparing the results of antibiotics with *Artemisia absinthium* extract, it became clear that some antibiotics, such as imipenem and gentamicin, showed higher efficacy in inhibiting bacterial growth compared to the plant extract. However, *Artemisia absinthium* extract exhibited significant antibacterial activity, especially when used at high concentrations.

These results indicate that plant extracts can be an important source of antimicrobial compounds and can be used as therapeutic supplements alongside conventional antibiotics. This is of great importance given the continued increase in bacterial resistance to antibiotics, which has become a global public health challenge (World Health Organization, 2020) [19–24].

Recommendations

Based on the results of this study, several recommendations can be made that may contribute to the development of future research and improve the use of medicinal plants in the medical and microbiological fields. Conduct further studies to identify the active chemical compounds in *Artemisia absinthium* that are responsible for its antibacterial activity.

Investigate the effect of *Artemisia absinthium* extract on other types of pathogenic bacteria to assess its efficacy as a broad-spectrum antimicrobial. Conduct advanced studies to determine the minimum inhibitory concentration (MIC) of *Artemisia absinthium* extract. Evaluate the synergistic effect between *Artemisia absinthium* extract and some conventional antibiotics to determine the possibility of using them together to enhance therapeutic efficacy [25–31].

CONCLUSIONS

The results presented in this study indicate that the hot water extract of *Artemisia absinthium* exhibited antibacterial activity that increased with increasing concentration. The 100% concentration recorded the highest inhibition zone diameter of 20 mm, indicating high efficacy in inhibiting bacterial growth. The 50% concentration recorded an inhibition zone diameter of 17 mm, indicating moderate antibacterial activity. The 25% concentration showed the least inhibitory activity with a diameter of 13 mm.

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