Vol.2 Issue.2 2026

ISSN : 3007-4738

# Inhibitory Effect of *Azadirachta indica* Extract and Antibiotics against Bacteria isolated from patients with Urinary Tract Infections

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**Article Information**
Received: 1-4-2026
Accepted: 21-4-2026
Published: 1-7-2026

## Abstract

**Background:** Urinary tract infection (Utls) are one of the most common bacterial infection worldwide, affecting millions of individuals annually and imposing a significant public health burden. The majority of (Utls) are caused by Urpathogenic *Escherichia coli*, although other pathogenic bacteria such as *Klebsiella penumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*. *Azadirachta indica* (Neem) is a versatile tree with multiple health benefits. Different parts of the tree were shown to exhibition antimicrobial effects against a wide variety of microbes. Screening of this medicinal plant for bioactive compounds may lead to progress of less expensive new antimicrobial agents with improved safety and efficacy. **Objective**: To isolate and identify bacteria responsible for urinary tract infections (UTIs) and evaluate the antibiotic susceptibility patterns of the isolated bacteria; and To assess the potential effectiveness of *Azadirachta indica* (Neem) leaf extract against UTI pathogens. **Material and methods**: **Methods:** A study was reached between October 1, 2025, to December 30, 2025. A total of 100 urine specimens from men and women aged 5 to 70 were carefully collected and examined, Bacterial isolates were diagnosed. This was confirmed with the use of HiCrome UTI agar media and the isolated bacteria were tested for antibiotic susceptibility using the Kirby-Bauer disk diffusion methods, following the standards set by the CLSI. Ethanolic and Aqueous extracts of Azadirachta indica were prepared at varying concentrations, which were applied on inoculated plates of Muller Hinton agar. The disc diffusion method was used to screen the antibacterial activity of both Azadirachta indica extract and synthetic antibiotics. **Results:** The most frequently identified organisms were as follows: 35.9% *Enterococcus faecalis* followed by 29.3% *Staphylococcus aureus*, 21.0% *E. coli* and only 6.6% have *pseudomonas aeruginosa* bacterial infection, all of which were multidrug-resistant (MDR). At all tested concentrations *Azadirachta indica* leaf extract exhibited significant antibacterial action against every bacterial species. **Conclusion:** Azadirachta indica (neem) leaf extract shown strong antibacterial activity against a range of bacterial pathogen strains.

**Keywords:** Urinary Tract Infections ; *Azadirachta indica* Leaf extract, antibiotics, Pathogenic bacteria,Green synthesis

## Introduction

Urinary tract infections (UTIs) are considered as the second most common type of an infection in humans and variety of pathogens could causing them, including the gram-positive and gram-negative bacteria. UTIs are the major health bother that affects the millions of people yearly in both a community and hospital setting worldwide (Al-Awkally et al., 2022). Urinary tract infection (Utls) are one of the most mutual bacterial infection worldwide, touching millions of individuals annually and imposing a significant public health burden .The majority of (Utls) are caused by Urpathogenic *E.coli*, although other pathogenic bacteria such as *K. penumoniae*, *P. aeruginosa*, and E. faecalis. can too be separation from clinical urine samples (Mancuso et al., 2023). The swelling prevalence of multidrug-resistant uropathogens has dared the efficiency of conventional antibiotic treatments ,principal to higher rates of treatment failure and reappearance (Wen et al., 2025). Normal products resulting as of medicinal plants have gained scientific interest outstanding to their bioactive complexes with inherent antimicrobial goods, offering a possible alternate or complementary strategy to synthetic (Kumar et al., 2025). Because therapeutic phytochemicals can accelerate the development of new medications, medicinal plant research and uses are growing daily. The majority of phytochemicals derived from plants, such as alkaloids, flavonoids, carotenoids, phenolic acids, tannins, saponins, and glucosinolates, have positive impacts on health and prevent cancer. Plants contain large amounts of phytochemicals, which are secondary aromatic plant metabolites that prevent disease. They are well known for their ability to prevent and lower the risk of chronic illnesses (such as cancer, heart disease, neurological disorders, etc.) and for their helpful mediation in the treatment of these conditions (Kashyap et al., 2022). Amongst these, *A. indica* (neem)is a well-studied therapeutic plant see for its diverse phytochemicals and antimicrobial possible against numerous bacterial pathogens (Ahmed et al., 2023). of India since this tree does not damage the environment and is free of pests and diseases. *A. indica* is considered as one of the most beneficial species for people. This neem tree is commonly referred to as Indian lilac or margosa. Neem is thought to be the most diverse and adaptable tree in the tropics, with enormous potential. Neem is a member of the Meliaceae family and is regarded as a botanical relative of mahogany (Agustina et al., 2018). The aim of this Isolation and identification bacteria from patients with UlT and Antibiotic susceptibility test . Evaluation of the antibacterial activity of neem leaf extract

## Materials and Methods

The present study is conducted between October 2025 and December 2025 in Iraq Al-Muthanna Maternity and Pediatric Hospital. Written consent was obtained from all the patients participating in this study. A total of 100 individuals with recurrent UTIs at various ages(46 men and 74 women)were included in this study. The study did not include patients with recognized risk factors for vesiocoureteral reflux or UTIs. At least three days before to the sampling, none of the study's participants had taken any antibiotics.

To diagnose a different species of bacterium, selective media are used. Based on the differences in the shape and color of the colonies, HiCrome UTI agar media makes it easier and faster to identify certain gram-positive and gram-negative bacteria. Gram stain was used to determine the morphological features of colonies. Muller-Hinton agar The agar disc diffusion method was used to test for antimicrobial sensitivity.

The fresh leafs of *A. indica* plant were collected Fallujah district in Anbar province in September and were classified by a botanist Dr. Mohamed Baqer Hussein Almosawi , Department of Biology, Al-Muthann university and washed with tap water firstly to remove all darts, then washed with distilled water three times and finally with deionized water, dry it with air. For prepared the two extractors about 25g from fresh leafs were cut to very small pieces and put in a beaker via 100ml from once from deionized water and the other from ethyl-alcohol, the solution was boiled for 30min. at 70C° in hot plate. The extractors were filtered two times after cooled, and the clearly extractors kept at 4C° for farther used.
To test antibacterial activity of *A. indica* leaf extract The aqueous and alcoholic extracts were prepared separately; the aqueous extract was dissolved in sterile deionized water, while the alcoholic extract was dissolved in ethanol. Equal concentrations of both extracts were then prepared (10mg),(15mg),(20mg),(25mg),(30mg) and their antibacterial activity was investigated using the agar etching method

**Ethical approval**

The principles of the Declaration of Helsinki served as the foundation for this inquiry. The questionnaire form was registered and the people's verbal consent was sought prior to collecting the samples. The study protocol was examined and approved by a local ethics commission.

**Statistical analysis**

SPSS (Social Statistical Package Science) version 24 is used for statistical analysis. To assess rates, the chi-square test is used with a significance level of $P < 0.01$.

## Results

A total of 100 urine samples were collected from patients suffering from urinary tract infection (UTI). The present results showed that 29 (29.0%) of UTI patients have single bacterial growth and 71 (71.0%) have mixed bacterial infection. Where these results showed that 65 out of 181 isolates (35.9%) have *E. faecalis* bacterial infection, followed by 53 isolates (29.3%) have *S. aureus* bacterial infection, 38 isolates (21.0%) have *E. coli* bacterial infection, 13 isolates (7.2%) have *k.pneumoniae* bacterial infection, and only 12 isolates (6.6%) have *p. aeruginosa* bacterial infection, as show in Figure (1).

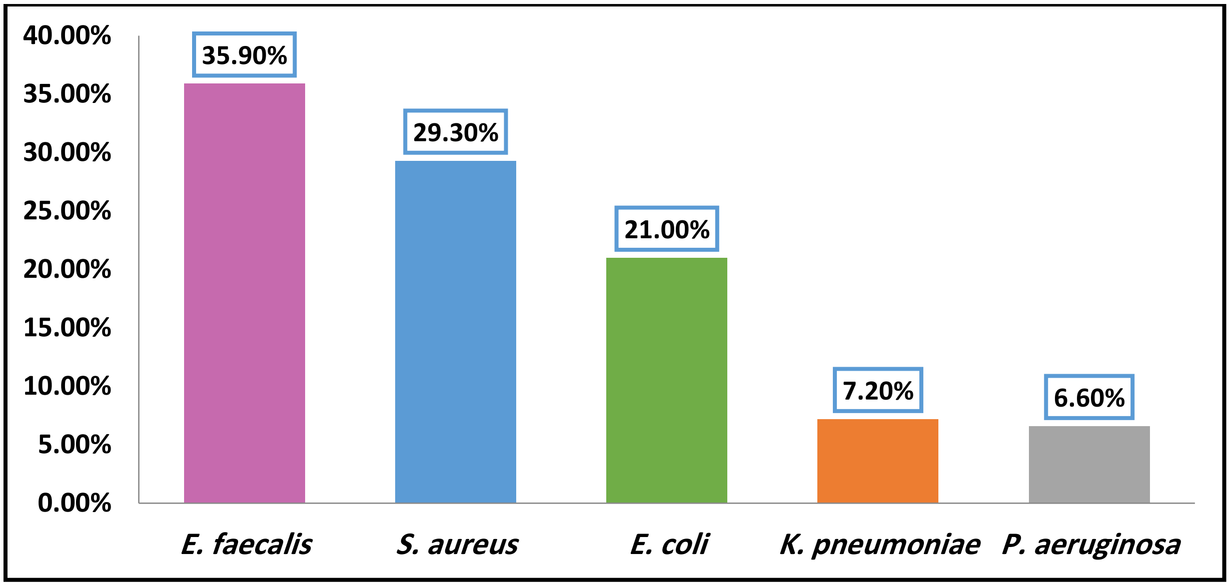
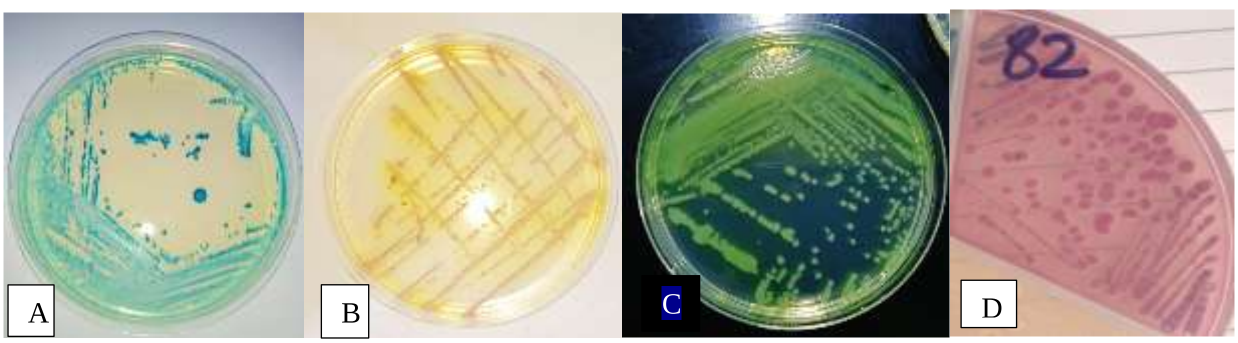



**Figure (1):** Percentages of bacterial species of UTI infection

The identification of some gram-positive bacteria and some gram- negative bacteria was based on the difference in the shape and color of the colonies on this agar media**.** *S.aureus* appeared as golden yellow isolated colonies, *E. faecalis* showed as small blue colonies, *E. coli* appearance is pink to purple, as shown in Figure (2), *P. aeruginosa* was colorless greenish.




**Figure(2):** Show the bacterial isolates on HiCrome UTI agar A. *E. Faecalis*. B. *S .aureus*. C. *P. aeruginosa*. D. *E.coli*

The present results show the females were more likely to get UTIs than males (69.0% versus 31.0%). The description for this difference amongst the incidence of males and females is that women are more liable to UTIs, and this reason stems from the datum that the urethra in women is briefer than in males, this means that the bacteria have to travel a shorter detachment before reaching the woman's bladder, which developments her risk of contagion (Barchitta et al., 2021). Therefore, through the existing study, it is concluded that the UTIs was determined by gender. the researchers explained the reason for the difference in these percentage in his study to the fact that hormonal changes during the menstrual round and pregnancy affect the acidity of the vagina and urine, which can enhance the growth of bacteria and increase the risk of infection, during pregnancy, the enlarged uterus puts pressure on the bladder and impedes its emptying, complete, which increases the risk of UTI. as show in Figure(3)

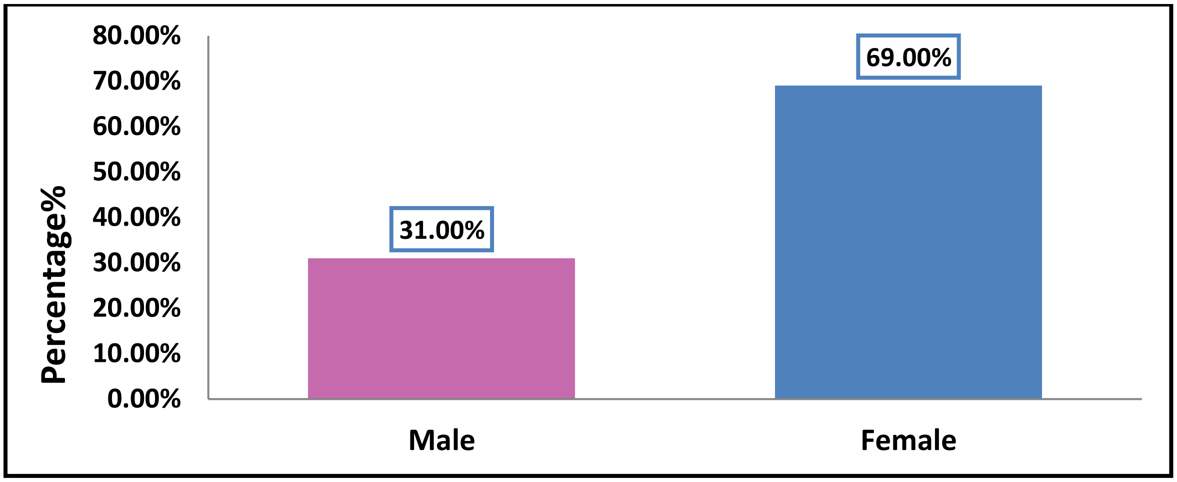



**Figure (3):** Gender distribution of patients suffering from UTIs infection.

**Antibiotics susceptibility**

Table (3) demonstrate the antibiotic susceptibility test for the isolates of different bacteria toward antibiotic. The study results manifested there are changes in the resistance and susceptibility confines between isolates of the bacterial that causing urinary tract infections(UTIS) including *S. aureus*, *E.faecalis, E. coli,* and *P. aeruginosa*. Therefore, this difference mediate the mutual influence of environmental factors, antibiotic use forms, and the genetic features of the bacterial isolate.

The isolates of *S. aureus* in this study displayed tall resistance to cephalosporins (75.4% ceftriaxone , 55.5%cefepime ), 84.9% tetracyclines, and 81% aztreonam, while exhibiting high susceptibility toward aminoglycosides (33.9amikacin), carbenems (60.3%imipenem, 33.9meropenem), and 37.7% piperacillin-tazobactam. These findings indicate the prevalence of resistance mechanisms that related to β-lactamase enzyme production, alterations in penicillin-binding proteins, and features common in methicillin-resistant strains. These outcomes are consistent with (Tong *et al*., 2015) who notified that staphylococcal resistance to β-lactam antibiotics is boosting globally, with aminoglycosides retaining relative efficacy. Moreover, align with (Kourtis et al., 2019) who suggested that the patterns of resistance are correlating with the overuse of cephalosporins. In disparity to these findings which reported by (Lee et al., 2021) reported about higher susceptibility rates toward tetracyclines. The difference that may be assigned to the variations in antibiotic prescribing patterns across regions.

*E. faecalis* isolates exhibited a clear resistance toward cephalosporins (66.1%ceftriaxone, 66.1%cefotaxime) and fluoroquinolones (46.1%ciprofloxacin, 69.2%ofloxacin). Whereas, exhibiting a better susceptibility to 61.5%amoxicillin-clavulanate, 61.5%imipenem, and 58.4% piperacillin-tazobactam. These findings are clarified by the innate resistance of Enterococcus to cephalosporins, as a result of their weak binding to PBP proteins. In addition to, mutations in the DNA gyrase enzyme responsible for the resistance to fluoroquinolone. These results are reliable with (Arias *et al*., 2021). who manifested the intrinsic resistance of Enterococcus to cephalosporins, also with (Hollenbeck and Rice, 2012) regarding to the increasing resistance toward fluoroquinolones. However, they contrast with (Santos et al., 2020), who demonstrated a higher sensitivity to ampicillin, which may be due to a variations in strains and isolation sources.

The study results showed that *E. coli* isolates showed a high resistance toward the third-generation of cephalosporins (92.1%ceftriaxone, 21% cefotaxime) and 78.9% amoxicillin-clavulanate. Additionally to, a significant resistance to fluoroquinolones, while showing high sensitivity to 63.1% imipenem, 13.1% meropenem, 21% amikacin, and 21% piperacillin-tazobactam. This pattern is assign to the prevalence of strains producing ESBL enzymes, which grant bacteria the ability to deactivate β-lactam antibiotics. These findings are compatible with (Flores-Mireles *et al*., 2015) who reported a global rise in *E. coli* resistance to cephalosporins, also with (Mancuso *et al*., 2021) who approved the efficacy of carbenems against ESBL-producing strains. These findings differ from those of (Majumder *et al*., 2022) who reported a higher sensitivity toward fluoroquinolones, a difference that may reflect regional variations in resistance patterns.

The study results demonstrated that isolates *of P. aeruginosa* showed a high resistance to cephalosporins (75% ceftriaxone, 58.3% cefotaxime), 58.3%amoxicillin-clavulanate, and 66.6% nalidixic acid, while showing better sensitivity to 66.6% piperacillin-tazobactam, 58.3% amikacin, and carbenems. This is described by a presence of the multiple resistance mechanisms in these bacteria, including the pumps of drug-excreting, reduced an outer membrane permeability, and the production of β-lactamase enzymes. These outcomes are consistent with a previous study conducted by (Fernández-Billón *et al*., 2023) who reported about a high resistance toward cephalosporins, as well as, with (Bassetti *et al*., 2018) regarding the efficacy of piperacillin-tazobactam and carbenems .However, they disagreement with a study introduced by (Karampatakis *et al*., 2025) which recorded about increase in a carbapenem resistance, which may mark a decrease in the prevalence of carbapenemase-producing strains in the studied area.

**Table (3) :** Antibiotic susceptibility test of different bacterial isolates toward antibiotic

| **Abbr.** | **Disc Content** | *S. aureus* N= 53 | *E. faecalis* N= 65 | *E. coli* N=38 | *P.aeruginosa* N=12 |
|---|---|---|---|---|---|
| **AT** | Azetronam 30mg | 43(81%) | 43(66.1%) | 36(94.7%) | 9(75%) |
| **CRO** | Ceftriaxone 30mg | 40(75.4%) | 43(66.1%) | 35(92.1%) | 9(75%) |
| **IPM** | Imipenem 10mg | 32(60.3%) | 40(61.5%) | 24(63.1%) | 3(25%) |
| **NA** | Nalidixic acid 30mg | 35(66%) | 40(61.5%) | 24(63.1%) | 8(66.6%) |
| **AMC** | Amoxillin-Clavulanate 30mg | 32(60.3%) | 40(61.5%) | 30(78.9%) | 7(58.3%) |
| **TE** | Tetracycline 30mg | 45(84.9%) | 45(69.2%) | 30(78.9%) | 7(58.3%) |
| **NX** | Norofloxacin 10mg | 48(90.5%) | 48(73.8%) | 28(73.6%) | 2(16.6%) |
| **CIP** | Ciprofloxacin 5mg | 20(37.7%) | 30(46.1%) | 8(21%) | 2(16.6%) |
| **OF** | Olfaxacin 5mg | 30(55.5%) | 45(69.2%) | 24(63.1%) | 2(16.6%) |
| **CAZ** | Cefotazidime 30mg | 30(55.5%) | 38(58.4%) | 30(78.9%) | 8(66.6%) |
| **COT** | TrimethoprimeSulfamethoxazole 25mg | 32(60.3%) | 40(61.5%) | 18(47.3%) | 2(16.6%) |
| **PIT** | Piperacillin-Tazobactam 100/10mg | 20(37.7%) | 38(58.4%) | 8(21%) | 8(66.6%) |

| | | | | | |
|---|---|---|---|---|---|
| **AK** | Amikacin 30mcg | 18(33.9%) | 40(61.5%) | 8(21%) | 7(58.3%) |
| **CFS** | CefoperazoneSulbactam75/30 mcg | 45(84.9%) | 45(69.2%) | 5(13.1%) | 7(58.3%) |
| **MRP** | Meropenem 10mg | 18(33.9%) | 23(35.3%) | 5(13.1%) | 6(50%) |
| **GEN** | Gentamycin 10mcg | 20(37.7%) | 23(35.3%) | 5(13.1%) | 6(50%) |
| **CPM** | Cefepime 30mcg | 30(55.5%) | 43(66.1%) | 8(21%) | 6(50%) |
| **CTX** | Cefotaxime 30mg | 30(55.5%) | 43(66.1%) | 8(21%) | 7(58.3%) |
| **LE** | Levofloxacin 5mg | 20(37.7%) | 30(46.1% | 8(21%) | 2(16.6%) |
| **P value** | | 0.001** ¥ | 0.001** ¥ | 0.001** ¥ | 0.001** ¥ |

¥: Chi-square test; **: significant at P <0.05;

**Antibacterial activity of leaf *A. indica* Extract against all tested bacteria**

The antibacterial activites of leaf extract aqueous and alcohol were evaluted against human pathogenic bacteria including *E. coli, S. aureus* , *E. faecalis* and *P. aeruginosa* .

As seen in Figures (5) and (6), the results unequivocally demonstrated that A. indica leaf extract was effective against various bacterial isolates. Any plant's antibacterial activity is dependent on the extraction circumstances, which include the kind and concentration of the solvent, the length of the extraction process, and the temperature. These variables all affect the kind and quantity of the active substance that is extracted.(Kadhim and Kadhim, 2012).

The antibacterial activites of leaf extract aqueous l against human pathogenic bacteria including *E. coli, S. aureus* , *E. faecalis* and *P.aeruginosa* .as Figure (5). The inhibition zone diameter of bacteria isolates increased as the concentration of extracts increased to reach of the extract significantly imporved as the concentration increased from (10mg/ml-30mg/ml).

At the maximum concentration (30 mg/ml), the extract demonstrated its maximum effectiveness at the maximum concentration of (30 mg/ml) the extract achieved inhibition zones of( 15 mm) for *E. faecalis*, *E.coli*, and *P. aeruginosa*. But, *S. aureus* appeared to be more resilient to the aqueous treatment, showing a maximum inhibition zone of only 10 mm.

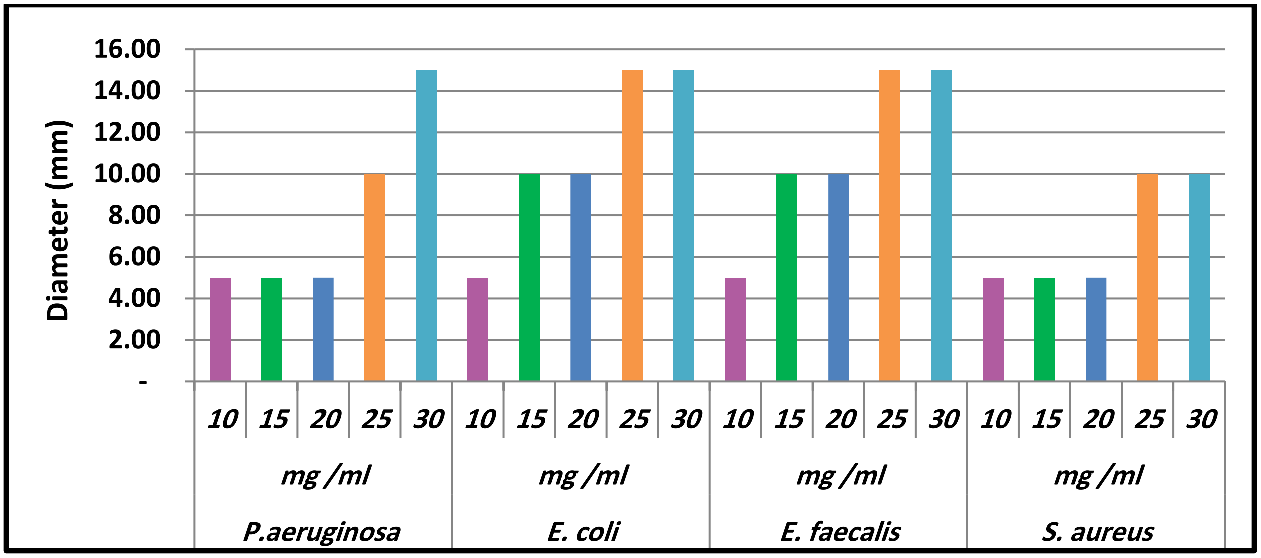



Figure (5): Diameters of the inhibition zones of aqueous extracts of neem leaves

The antibacterial activites of leaf extract alcohol against human pathogenic bacteria including *E. coli, S.aureus* , *E. faecalis* and *P. aeruginosa* .as Figure (6). The inhibition zone diameter of bacteria isolates increased as the concentration of extracts increased to reach of the extract significantly imporved as the concentration increased from (10mg/ml-30mg/ml).

At the maximum concentration (30 mg/ml), the extract demonstrated its maximum effectiveness, resilient inhibition zones of 20 mm for *E. faecalis*, *E. coli,* and *P.aeruginosa.* Grippingly, while *S. aureus* showed a relatively lower vulnerability at initial concentrations, it reached a notable inhibition zone of (15 mm) at the concentration( 30 mg/ml) .

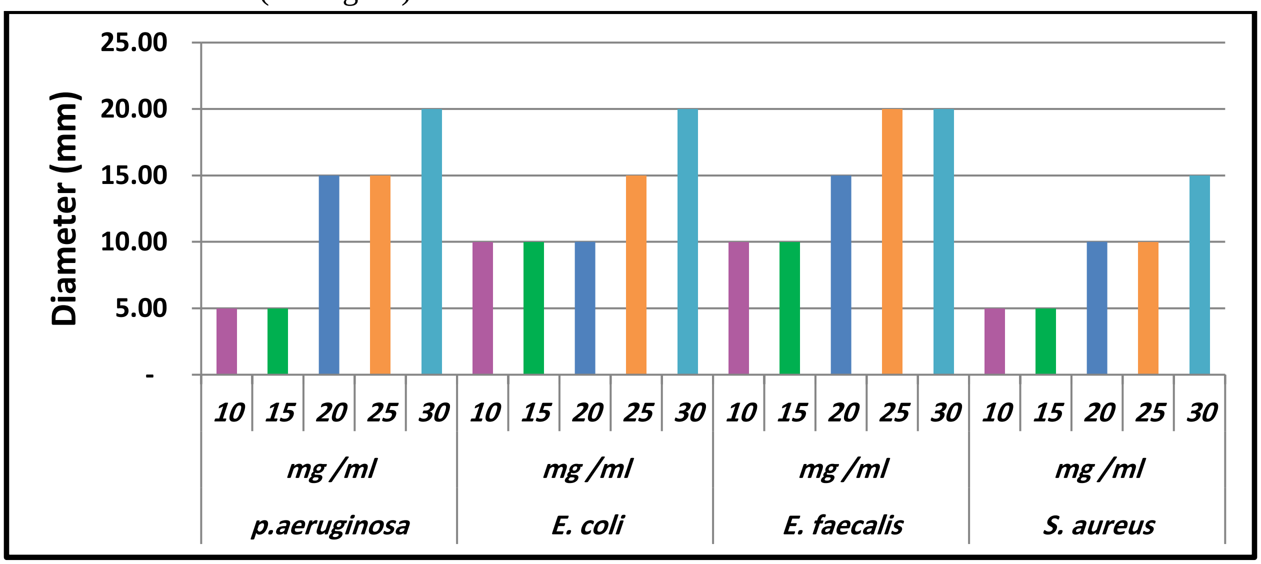



Figure (6): Diameters of the inhibition zones of alcoholic extracts of neem leaves

## Discussion

Both Gram-positive and Gram-negative bacteria can cause urinary tract infections, which are among the most prevalent infectious diseases in the world. Finding the right therapy for the infection that results from the spread of these MDR bacterial strains, which represent a serious risk to the health of people of all ages, depends on research on bacterial resistance to multiple antibiotics. Most persons are prone to urinary tract infections because of changes in a range of anatomical and physiological characteristics. In this study, we found that females had greater infection rates (69.0%) compared to males (31.0%). E. faecalis (65) was the most frequently occurring pathogen in the urine isolation, followed by S. aureus (53), E. coli (38), and P. aeruginosa (12).

Approximately 90% of UTI patients worldwide are infected with E. coli, which is thought to be one of the primary causes of UTIs (Mouhammed and Gdoura, 2024), The study by Jaaz and Hamim (2014) in the city of Al-Qadisiyah, where the percentage of bacterial isolates from UTIs reached (%39.06), had a higher incidence of UTIs than previous local, Arab, and international studies. Additionally, according to Shuwaikh and Jassim's 2014 study, the percentage of bacteria isolated from urine samples in hospitals in Baghdad and its environs was 41.6%.

In addition to taking antibiotics before to sample collection, the number of samples and variations in geographic and hygienic settings account for the high percentage of infections. It might be because bacteria other than E. faecalis bacteria have adapted to thrive in the urinary system and are tolerant of different environmental conditions. They are more likely to cause infection due to their suitability as well as the existence of obvious and potent adverse elements.

53 isolates (29.3%) of UTI patients had a S. aureus bacterial infection; this is greater than the findings of both (Mhana et al., 2023), and the Dari, (2019) study, where the corresponding percentages were 8.5% and 10.52%.

In contrast to the findings of a study by Ghaima and Tamara (2022), which revealed that the percentage of UTIs with K. pneumoniae reached 29.2%, the current study demonstrated that the percentage of UTIs with K. pneumoniae was 13 isolates (7.2%). Additionally, it was lower than Olukemi et al. (2023) at Niger Delta University Teaching Hospital, where 30% of all urine samples had bacterial isolates of K. pneumoniae. This discrepancy in isolation rates could be attributed to variations in sample collection techniques, locations, and times.

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The microbiota of the urine changes with age, including microbial communities in other physiological environments (Colella et al., 2023) Children and adults have different microbiome communities as a result of urinary metabolites from lifestyle changes, personal hygiene, and voiding habits. Adults are found to have several bacterial genera in various age groups (Tang et al., 2017). According to researches, UTIs is more common in middle aged and older peoples than in younger.

The statistical analysis's findings demonstrated that the concentrations varied significantly. where the inhibition zone diameter is significantly greater at (30 mg/ml) than at other concentrations. The extraction of A. indica leaves using several solvents revealed notable variations between them. When compared to other solvents, alcohol extract significantly increased the diameter of the inhibitory zone. Although other mechanisms have been proposed, such as the inhibition of microbial enzymes, the impact on membranes, or the deprivation of substrates necessary for microbial development, the mode of action of these compounds, like polyphenols, is typically linked to polyphenol-protein interactions (Janecki and Kolodziej, 2010).

In this study, we have shown that ethanolic and aqueous extracts of A. indica (neem) leaves exhibit potent antibacterial activity against every tested bacterial strain at every dosage. The results of other studies on the antibacterial properties of neem leaf extract were almost exactly the same as ours. Among these investigations (Mohammed et al., 2015) They claimed that the crude extract of the neem plant was highly efficient against P. aeruginosa, E. coli, S. aureus, and Staphylococcus aureus. After 24 hours, they discovered that an extract concentration of 10 mg/ml had considerably decreased S. aureus, whereas extracts with higher concentrations eliminated all live germs in a shorter amount of time.

Numerous research have shown that the ethanol extract of plant parts was more inhibitory than the aqueous extract, and this study supports the findings of those who found that neem leaf reduced the growth of some bacterial infections. This is because ethanol is a superior extractant. (Chibuzo, 2019). This study is also in agreement with several studies (Bachabi et al., 2018) (Melo et al., 2021). The test organisms were significantly impacted by the neem leaf aqueous extract. The antibacterial activity of A. indica leaf aqueous extracts against human pathogenic bacteria was compared by Raja and his associates. At every tested concentration, they discovered that leaf extract demonstrated strong antibacterial action against these microorganisms.

This study is also in agreement with (George et al., 2016)(Ali et al., 2021), who stated that the leaf of this plant has inhibitory effect on *P. aeruginosa*. The outcome shown that ethanol extract outperformed other extracts in its ability to combat the test organisms. The results of the investigation demonstrated a good comparison between the plant extracts and the standard antibiotics utilized as a positive control. It is thought that the phytochemicals in this plant are what give it its antibacterial properties. shown that considerable antibacterial activity in A. indica leaves may be caused by high amounts of azadirachtins, querectin, and β-sitoeterol. Alkaloids, glycosides, tannins, and saponins are among the phytochemicals found in A. indica, according to this study. Therefore, these are the plant's defense mechanisms against infections. According to this study, A. indica leaf extract can be utilized as a medicinal substance.(Chibuzo, 2019)

**Conclusion**

This study showed that bacteria from individuals with UlT infections with varying patterns of antibiotic susceptibility may be successfully isolated and identified. A variety of bacterial pathogen strains were susceptible to the antibacterial activity of A. indica (neem) leaf extract.

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