

## Detection of the Effect of a Natural Product *Syzygium aromaticum* on Bacteria Causing Urinary Tract Infections Isolated from Patients at Tikrit Teaching Hospital

Maream Kaream Khalaf Al-Hallawi<sup>1\*</sup>, Firas Adnan Hussein<sup>2</sup>

1, 2, Dept. of Biology / College of Education for Girls/ Tikrit University-Iraq

\*Corresponding author's email: [Maream.khalaf484@st.tu.edu.iq](mailto:Maream.khalaf484@st.tu.edu.iq)

[Email addresses of coauthors: firas.adnan@tu.edu.iq](mailto:firas.adnan@tu.edu.iq)

### Abstract

108 samples were collected from patients suffering from urinary tract infections at Tikrit Teaching Hospital. Bacterial growth testing was performed on the studied samples. The bacterial species were isolated and identified, activated on special culture media, and treated with aqueous and alcoholic extracts of clove. The extracts were prepared, and their active components were analyzed using GC-MS. Three concentrations of each extract were prepared and tested against the bacterial isolates to determine the inhibition diameters. The data were collected and statistically analyzed. The results of the bacterial diagnosis showed that bacterial pathogens were the most prominent factor in the infections, with *Escherichia coli* and *Klebsiella species* bacteria recording the highest prevalence rates among the isolated groups. Treatment of the isolated species with aqueous and alcoholic clove extract proved the efficacy of the alcoholic extract, which showed a clear advantage in destroying bacterial cells. The inhibition diameters peaked at a concentration of 750 mg/ml, reaching 30 mm against *Escherichia coli* and 28 mm against *Enterococcus faecalis*. The efficacy of the aqueous extract: The aqueous extract achieved a maximum inhibition diameter of 22 mm against *Enterococcus faecalis* at a concentration of 750 mg/ml. Clove extracts (both aqueous and alcoholic) exhibited clear inhibitory effects against the bacteria causing these infections. The alcoholic extract contains eugenol (86.25%) supported by caryophyllene (11.20%), giving it a high efficacy. The aqueous extract contains long-chain carbonic acids (diisooctyl phthalate and docosanoic acid) exceeding 66%.

**Key words:** UPEC, *Syzygium aromaticum*, Eugenol, GC-MS analysis, Agar well diffusion.

### Introduction

Urinary tract infections (UTIs) are a major global health problem, second only to respiratory infections, and a leading cause of morbidity and mortality worldwide [1]. UTIs encompass a wide range of symptoms, including cystitis, pyelonephritis, septicemia, and catheter-associated UTIs. Bacteria are the most common cause of UTIs, while fungi and viruses are less common [2].

Bacteriuria is defined as a condition in which the number of bacteria in the urine is greater than  $10^5$  colony-forming units per milliliter [3]. It is often caused by bacterial diseases originating from the gastrointestinal tract. In community-acquired urinary tract infections (UTIs), *Escherichia coli* and *Klebsiella species* predominate [4]. Hospital-acquired UTIs involve a wider range of pathogens, such as *Escherichia coli*, *Staphylococcus*, *Enterococcus*, and others [5]. However, *Escherichia coli* remains the most common

urinary tract infection, and antibiotic-resistant strains have emerged [6].

Urinary bacterial isolates develop resistance to antibiotics in three ways: intrinsic resistance, acquired resistance, and adaptive resistance [7]. In the urinary tract, certain types of bacteria, such as *Proteus mirabilis* and *P. aeruginosa*, form a biofilm and hide within it, living inside the biofilm in a relatively protected environment [8]. Unfortunately, the spread of antibiotic resistance has made treating urinary tract infections increasingly difficult. To address this worrying situation, research teams are continuously evaluating the use of antibiotic alternatives [9], such as medicinal plants. Medicinal plants have been used for thousands of years to treat various diseases, including bacterial infections [10]. Research has indicated that plant extracts contain bioactive compounds that disrupt bacterial cellular processes [11]. Herbal medicines consist of parts of the plant such as fruits,

flowers, leaves, seeds, and roots. Clove buds have also emerged as an alternative treatment option [12].

*Syzygium aromaticum*, also known as clove, belongs to the Myrtaceae. Cloves are characterized by potent antimicrobial properties. The flower buds of the clove plant contain high concentrations of essential oil. Cloves have garnered significant attention due to their antimicrobial and cytotoxic properties. They are also a major plant source of phenolic compounds, such as flavonoids and hydroxyl benzoic acids. Eugenol is the main compound found in cloves and has been used for its anti-inflammatory properties [13]. The current study aimed to evaluate the efficacy of alcoholic and aqueous extracts of clove buds against certain bacterial species isolated from urinary tract infections and to explore their potential use as natural alternatives to conventional antibiotics used in the treatment of these infections. identifies both Gram-negative and Gram-positive bacteria. The system was used to confirm the types of isolates using a diagnostic kit provided by the manufacturer. Each isolate requires its own card, which contains 64 wells. Each well contains a dried indicator medium. The system records the indicators and changes, allowing for sample identification according to the manufacturer's instructions.

**Preservation and Maintenance of Bacterial Isolates:** After identification, bacterial isolates were preserved on slanted culture media of nutrient agar at 4°C. Their purity was confirmed by monthly culture renewal throughout the study period. For long-term preservation of isolates, a test tube containing 5-10 ml of nutrient broth was inoculated with a single colony. The culture was incubated for 24 hours, and 0.8 ml of the culture was transferred to tightly

## Material and Methods

**Sample Collection:** Study samples were collected between September 2025 and December 2025 from patients with urinary tract infections or symptoms at Tikrit Teaching Hospital. The study included patients aged 10 to 80 years. A total of 108 urine samples were collected, of which 78 were from patients suspected of having a urinary tract infection based on the diagnosis of a specialist physician.

**Isolation and Identification of Bacterial Isolates:** Samples collected from patients were cultured directly onto MacConcy Agar and Blood Agar media prepared according to the manufacturer's instructions. These media were then incubated at 37°C. Growth was observed after 24 hours, and the samples were identified using the Vitek2 Compact System. This system

sealed bottles containing 0.2 ml of sterile glycerol. The mixture was mixed by inverting the tube several times after sealing it, and the culture was stored at -20°C .

**Preparation of plant extracts:** Plant extracts were prepared for both cloves. About 50 grams of each were taken and cold-ground. Then, they were soaked in distilled water and ethyl alcohol. 25 grams were soaked in 500 ml of distilled water and 25 grams in 500 ml of ethyl alcohol. After that, they were shaken every 3 hours for 4 days. Then, large impurities were removed using pieces of gauze and then filter paper to ensure a pure extract was obtained. The extracts were distributed in glass dishes and left to air dry. Then, the extracts were collected in tightly sealed glass bottles [14].

**Sample analysis using the Shimadzu GCMS-QP2010 Plus:** The device's injector was set to 2 microliters in Split mode to avoid saturating the column with the sample. A 30-meter Inert Cap 1 (a non-polar column bonded with 100% dimethylpolysiloxane) capillary column was used, with helium (99.999% purity) as the carrier gas and a flow rate of 13.6 mL/min. The oven's thermal program was started at 100°C and held for one minute. The temperature was then increased by 17°C to 150°C, held for four minutes, and then increased by 9°C to 250°C, held for one minute, for a total retention time of 20 minutes.

**Preparing Extract Concentrations:** A concentration of 750 mg/ml is prepared by dissolving 7.5 grams of 10 ml of solvent. A concentration of 500 mg/ml is prepared by dissolving 2 ml of the 750 mg/ml solution and adding 1 ml of pure solvent, resulting in 3 ml of a 500 mg/ml solution. To obtain a concentration of 250 mg/ml, a certain volume of the 500 mg/ml solution is taken, for example, 1 ml, and an equal volume of

pure solvent, 1 ml, is added to form a 250 mg/ml solution[15].

### Treatment Methods

#### First: Activation of Bacterial Cultures:

The bacterial isolates used were activated by culturing them on nutrient agar medium to reactivate the colonies and ensure accurate results during testing. The plates were then incubated for 24 hours, making them ready for use in subsequent experiments.

**Second: Treatment of Bacterial Isolates with Extracts:** The bacterial isolates were treated with both the aqueous and alcoholic extracts of clove, using three different concentrations of each, as detailed in Table 1.

#### Third: Recording Results and Statistical Analysis:

The results relating to the detection of the effect of the extracts (aqueous and alcoholic) on inhibiting or growing the isolates were recorded. Statistical analysis of the obtained data was then performed to assess the significance of the differences and results.

**Treatment of isolates with extracts:** The effectiveness of the compounds against bacterial isolates was assessed using Mueller-Hinton Agar medium, which was prepared according to the manufacturer's instructions. The bacterial suspension was then cultured and diffused using a glass diffuser. After that, 5 mm diameter holes were made using a cork drill, and the concentrations were distributed according to Table (1) or the dilutions. The medium was incubated in an incubator at a temperature of 37 for 24 hours. After that, the inhibition diameter was measured using a ruler [16].

Table (1): Bacterial isolates, types of extracts, their concentrations, and their codes.

|     | <i>Klebsiella pneumoniae</i> | <i>Escherichia coli</i> | <i>Staphylococcus haemolyticus</i> | <i>Staphylococcus aureus</i> | <i>Enterococcus faecalis</i> | <i>Pantoea .spp</i> |
|-----|------------------------------|-------------------------|------------------------------------|------------------------------|------------------------------|---------------------|
| 257 | 257                          | 257                     | 257                                | 257                          | 257                          | 257                 |
| 505 | 505                          | 505                     | 505                                | 505                          | 505                          | 505                 |
| 123 | 123                          | 123                     | 123                                | 123                          | 123                          | 123                 |
| 257 | 257                          | 257                     | 257                                | 257                          | 257                          | 257                 |
| 505 | 505                          | 505                     | 505                                | 505                          | 505                          | 505                 |
| 123 | 123                          | 123                     | 123                                | 123                          | 123                          | 123                 |

**Statistical Analysis:** The results were statistically analyzed using the Minitab Ver.17 statistical software by applying the F-test and Chi-square test. The means were compared using Duncan's multiple range test.

## Results and discussion

**Detection of bacterial infections:** The results of the examination of 108 urine samples, as shown in Table (2), for bacterial infections related to urinary tract infections (UTIs), revealed that 83 samples (76.85%) were positive for bacterial growth, while 25 samples (23.14%) were negative. These results indicate that the

**Bacterial types of infections:** In the Gram-positive samples, bacteria were classified as Gram-negative or Gram-positive. The Gram-negative group comprised the largest number of isolates, as shown in Table (3), and included the following species: *Escherichia coli* (40

vast majority of the samples contained bacterial growth.

The high percentage of positive growth (76.85%) is attributed to the accuracy of the initial clinical diagnosis of patients and is consistent with global studies that classify urinary tract infections as one of the most common bacterial infections. The remaining 23.14% of samples that did not show bacterial growth can be explained by several scientific reasons, including the patient's use of antibiotics, the presence of a non-bacterial causative agent such as viruses, fungi, or parasites, or the presence of difficult-to-cultivate bacteria requiring special culture media. These infection rates were consistent with those of [17].

| Total number of samples | Positive growth |            | Negative growth |            |
|-------------------------|-----------------|------------|-----------------|------------|
|                         | Number          | Percentage | Number          | Percentage |
| 108                     | 83              | 76.85%     | 25              | 23.14%     |

Table (2) Distribution of positive and negative bacterial growth infections

isolates), *Klebsiella pneumoniae* (21 isolates), and *Pantoea* (1 isolate). The Gram-positive isolates were distributed across several genera and species, including: *Enterococcus faecalis* (17 isolates), *Staphylococcus haemolyticus* (13 isolates), *Staphylococcus epidermidis* (11

isolates), *Staphylococcus aureus* (8 isolates), and *Enterococcus faecium*

Table (3) Distribution of bacterial infections among positive urinary tract infections

| isolated bacteria                  | Gram stain | Number of isolates | percentage %   |
|------------------------------------|------------|--------------------|----------------|
| <i>Escherichia coli</i>            | -          | 40                 | 34.78%         |
| <i>Klebsiella pneumonia ssp</i>    | -          | 21                 | 18.26%         |
| <i>Staphylococcus aureus</i>       | +          | 8                  | 6.95%          |
| <i>Enterococcus faecalis</i>       | +          | 17                 | 14.78%         |
| <i>Staphylococcus haemolyticus</i> | +          | 13                 | 11.30%         |
| <i>Staphylococcus epidermis</i>    | +          | 11                 | 9.56%          |
| <i>Enterococcus faecium</i>        | +          | 4                  | 3.48%          |
| <i>Pantoea spp</i>                 | -          | 1                  | 0.86%          |
| <b>the total</b>                   | <b>115</b> | <b>115</b>         | <b>100.00%</b> |

The results showed that *Escherichia coli* was the most prevalent bacterium, with 40 isolates, a finding consistent with previous studies confirming that this species causes approximately 70-80% of urinary tract infections. This is attributed to its possession of specialized virulence factors, such as P-fimbriae cilia, which enable it to adhere to bladder epithelial cells and resist the normal flow of urine.

*Klebsiella pneumoniae* ranked second with 21 isolates, a finding corroborated by numerous clinical studies that place *Klebsiella pneumoniae* as a common second choice in hospital-acquired infections. Among Gram-positive bacteria, *Enterococcus faecalis* accounted for 17 isolates, along with several *Staphylococcus* species. This aligns with recent trends in microbiological studies indicating the increasing role of Gram-positive bacteria as significant pathogens, particularly in chronic infections or in patients who have undergone urinary catheterization [18].also identified the same pathogen with a similar number of isolates.

#### Chromatographic Analysis of Alcoholic

**Clove Extract:** The chromatographic analysis of the alcoholic clove extract divided the compounds into two groups: phenolic and resinous. The phenolic compounds were more prevalent than the resinous ones. Phenols are known for their medicinal properties. In this sample, 4-Allyl-6-methoxyphenol constitutes 8625% of the basic phenol, while 4-(2-propenyl)-phenol appears at 0.36%. Methyl salicylate, which is strophanol, is present at 0.05%. Regarding resins and terpenes, caryophyllene, also known as sesquiterpene, is the main resin structure in this sample and is present at 7.74%. Caryophyllene oxide, a primary product of terpene oxidation and often found in resinous components, is present at 1.12%. Alpha-farnesene is also a resinous compound, present at 1.08%. Copaene is another resinous compound present. Resins are present at a rate of 0.06%, while the other materials are fatty acids and hydrocarbons.

Analysis of the alcoholic extract of the clove plant, *Syzygium aromaticum*, reveals a high concentration of phenolic and terpene compounds of significant medicinal value. Analysis of the chemical constituents shows that the extract contains 18 major constituents, with the most important and dominant being eugenol (Allyl-6-methoxyphenol/Eugenol 3). Constituting 86.25% of the extract, this phenolic compound (the primary active ingredient in clove) acts as a bactericidal agent through specific mechanisms. Caryophyllene, the seventh constituent (7.74%), is classified as a sesquiterpene and is responsible for its anti-inflammatory properties. Other important compounds include caryophyllene oxide, the ninth constituent (1.08%). Methyl salicylate, at peak 1, is a pain reliever similar to aspirin. Fatty and resinous acids, such as tetracosanoic acid, are present at peaks 17 and 18. Due to the absolute dominance of eugenol, this extract possesses broad-spectrum antimicrobial properties, destroying the cell membranes of bacteria and fungi. The resinous and terpenoid compounds, such as caryophyllene, copaene, and caryophyllene oxide, contribute to the oily texture and facilitate the penetration of phenolic compounds into plant tissues. They also play a significant role as plant defense compounds.

This extract is considered "very pure" and of high quality because the eugenol content exceeds 85%, making it an excellent candidate for use as a natural antibacterial agent or in pharmaceutical applications.

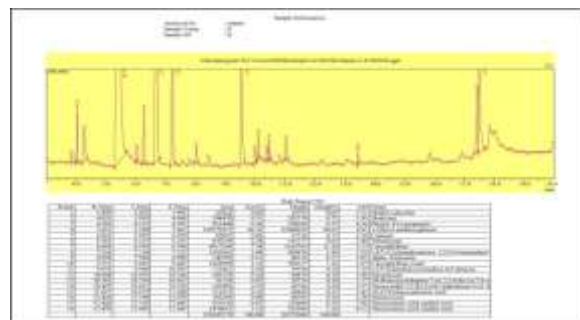


Figure (1): Chromatographic examination of alcoholic clove extract

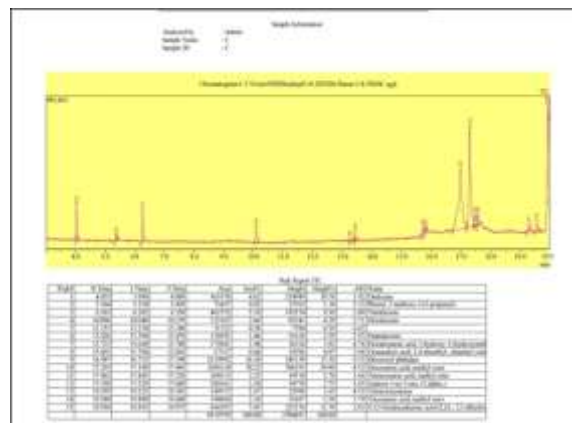
**Chromatographic Analysis of Aqueous Clove Extract:** Figure (2) shows the chromatographic analysis of the extract. The compounds were divided into major groups, including phthalate esters, fatty acids, and some alkane hydrocarbons. Phthalates were the most abundant in this sample, outperforming the other components in terms of concentration and chromatographic area. Diisooctyl phthalate was the primary and dominant component, constituting a very high percentage of 36.10% at a retention time of 16.987 minutes. It was followed in importance and percentage by docosanoic acid methyl ester, which appeared at 30.23%. This is a long-chain ester that imparts an oily character to the sample. As for unsaturated fatty acids, 9,12-octadecadenoic acid (Z,Z), also known as linoleic acid, stood out at 7.47%. This is a biologically important compound that indicates the nutritional or therapeutic properties of the sample. The analysis also revealed the presence of a series of saturated hydrocarbons, such as tetradecane (5.19%) and hexadecane (2.60%), which contribute to the overall chemical structure of the extract. Phenolicly, 2-methoxy-3-(2-propenyl)- was found at 0.82%. Despite its low concentration, it is a highly biologically active compound. The remaining components consisted of secondary fatty acids and various esters, such as tricosanoic acid methyl ester (2.18%) and other compounds ranging

from 0.5% to 2.5%, reflecting the wide chemical diversity of the extract's composition [14].

Chromatographic analysis of the aqueous extract of cloves reveals a significant difference in the nature and proportions of the extracted compounds compared to the previously obtained alcoholic extract. Here, heavier compounds and fatty acids appear at higher concentrations. Analysis of the most important peaks shows that the aqueous extract contains 15 major peaks, the most significant being peak number 9 (Diisooctyl phthalate): 36.10%, the highest concentration in this extract. This compound often appears as a plasticizer, and its high concentration may indicate interference from the equipment used or that it is a byproduct of the aqueous extraction process.

Peak number 10 is Docosanoic acid, methyl ester, at 30.23%. This is an ester of the long-chain fatty acid Behenic acid. Peak number 15, 9,12-Octadecadienoic acid (Z,Z-), at 7.47%, is classified as a fatty compound related to linoleic acid. As for the third peak, Tetradecane, which constitutes 5.19%, it is classified as an alkane hydrocarbon. Regarding phenolic and resinous substances, we observe a significant decrease in the percentage of eugenol, the main phenolic compound in clove, in the aqueous extract compared to the alcoholic extract. This is because eugenol is an oily compound that dissolves better in alcohol than in water.

Figure (2): Chromatographic examination of aqueous clove extract



The aqueous extract possesses the following properties: it is less antibacterial than the alcoholic extract due to its lower phenol content; and it has anti-inflammatory properties thanks to the presence of steroid compounds and unsaturated fatty acids.

In the alcoholic extract, the active ingredient was eugenol at 86%, making it a very potent antibiotic. In the aqueous extract, the fatty acids and esters predominate, making it less lethal to bacteria.

Effect of Clove Extract on Bacteria: The results for clove extract showed that the alcoholic extract was more effective than the aqueous extract, with an average inhibition diameter of 18.39 A compared to 12.44 B for the aqueous extract. The inhibition diameter also increased with increasing concentration, with the alcoholic extract recording the highest average at 23.33 A at 75% concentration. *Staphylococcus aureus* was the most affected isolate with an overall average of 23.5, followed by *Enterococcus faecalis* with an average of 20.33. These results are consistent with recent studies showing that clove is rich in active phenolic compounds such as eugenol and caryophyllene, and that these compounds may affect the bacterial cell membrane and cause loss of cellular components. Furthermore, alcoholic solvents often extract a larger quantity of active compounds compared to

water, which explains the higher inhibition diameter in the alcoholic extract and its increase with concentration, as demonstrated by [19].

This table presents an evaluation of the inhibitory efficacy of aqueous and alcoholic clove extracts at different concentrations against a range of bacterial pathogens isolated from urine samples. For the alcoholic clove extract, the table shows a clear superiority in inhibition diameters compared to the aqueous extract at all concentrations (250, 500, and 750). This is

attributed to ethanol's ability to extract biologically active phenolic compounds, primarily eugenol, at higher concentrations. These compounds are hydrophobic, resulting in greater solubility in alcoholic solvents and thus increasing their ability to penetrate and destroy the bacterial lipid cell membrane. The aqueous clove extract, however, exhibited lower efficacy, with some concentrations, such as 500, recording lower inhibition diameters. This suggests that the aqueous solvent did not adequately extract the oily and volatile components.

**Table (4) Effect of aqueous and alcoholic extracts of the clove plant**

| Extract type                              | % the focus | Bacterial isolates     |                         |                       |                       |                     |                        | Average concentration | Average extract |
|-------------------------------------------|-------------|------------------------|-------------------------|-----------------------|-----------------------|---------------------|------------------------|-----------------------|-----------------|
|                                           |             | <i>Klebsiella</i><br>a | <i>Escherichia coli</i> | <i>Staphylococcus</i> | <i>Staphylococcus</i> | <i>Enterococcus</i> | <i>Pantoea</i><br>.spp |                       |                 |
| Water cloves                              | 250         | k 5                    | k 5                     | jk 6                  | cd 18                 | d 17                | h 9                    | E 10.00               | B12.44          |
|                                           | 500         | k 5                    | ij 7                    | hi 8                  | b 23                  | c 19                | g 11                   | D 12.17               |                 |
|                                           | 750         | k 5                    | h 9                     | f 13                  | a 27                  | b 22                | e 15                   | C 15.17               |                 |
| Average inhibition in the aqueous extract |             | 5.00<br>f              | 7.00<br>e               | 9.00<br>d             | 22.67<br>a            | 19.33<br>b          | 11.67<br>c             |                       |                 |
| alcoholic clove                           | 250         | i 5                    | e 21                    | i 5                   | e 20                  | f 17                | g 15                   | D 13.83               | A18.39          |
|                                           | 500         | h 9                    | d 25                    | h 9                   | d 25                  | e 20                | e 20                   | B 18.00               |                 |
|                                           | 750         | g 14                   | a 30                    | g 15                  | b 28                  | bc 27               | cd 26                  | 23.33 A               |                 |

|                                             |           |            |           |            |            |            |  |
|---------------------------------------------|-----------|------------|-----------|------------|------------|------------|--|
| Average inhibition in the alcoholic extract | 9.33<br>c | 25.33<br>a | 9.67<br>c | 24.33<br>a | 21.33<br>b | 20.33<br>b |  |
| Overall average of isolates                 | 7.17<br>e | 16.17<br>c | 9.34<br>d | 23.5<br>a  | 20.33<br>b | 16.00<br>c |  |

(a, b, c...) indicates statistically significant differences ( $P < 0.05$ ). The letter "a" at a concentration of 750 is the best inhibitory treatment.

## Conclusion

The results showed that bacterial infections are the primary cause of urinary tract infections (UTIs) among the patients included in the study. The predominant bacterial species, *Escherichia coli* and *Klebsiella*, are the most common and leading causative agents of UTIs. The study demonstrated that clove extracts (both aqueous and alcoholic) have clear and significant inhibitory effects against the bacteria causing these infections. In vitro analyses revealed that the alcoholic clove extract was significantly superior to the aqueous extract in terms of efficacy and effectiveness in all treatments. The alcoholic extract in this sample is of high quality and purity, containing a very high percentage of eugenol (86.25%) supported by caryophyllene (11.20%), giving it excellent therapeutic and biological efficacy.

The aqueous extract in this sample is predominantly composed of fatty acids, esters, and long-chain carbonic acids (such as diisooctyl phthalate and docosanoic acid), which together constitute over 66% of the total composition.

**Acknowledgment:** We are grateful to .....Funding sources should be acknowledged.

## References

- [1] He, Y., Zhao, J., Wang, L., Han, C., Yan, R., Zhu, P., ... & He, W. (2025). Epidemiological trends and predictions of urinary tract infections in the global burden of disease study 2021. *Scientific reports*, 15(1), 4702.
- [2] Gupta, K., Hooton, T. M., & Stamm, W. E. (2005). Isolation of fluoroquinolone-resistant rectal *Escherichia coli* after treatment of acute uncomplicated cystitis. *Journal of antimicrobial chemotherapy*, 56(1), 243-246.
- [3] Wagenlehner, F., Wullt, B., Ballarini, S., Zingg, D., & Naber, K. G. (2018). Social and economic burden of recurrent urinary tract infections and quality of life: a patient web-based study (GESPRIT). *Expert review of pharmacoeconomics & outcomes research*, 18(1), 107-117.
- [4] Alpay, Y., Aykin, N., Korkmaz, P., Gulduren, H. M., & Caglan, F. C. (2018). Urinary tract infections in the geriatric patients. *Pakistan journal of medical sciences*, 34(1)67
- [5] Donkor, E. S., Horlortu, P. Z., Dayie, N. T., Obeng-Nkrumah, N., & Labi, A. K. (2019). Community acquired urinary tract infections among adults in Accra, Ghana. *Infection and Drug Resistance*, 2059-2067.
- [6] Chelkeba, L., Fanta, K., Mulugeta, T., & Melaku, T. (2022). Bacterial profile and antimicrobial resistance patterns of common bacteria among pregnant women with bacteriuria in Ethiopia: a systematic review and meta-analysis. *Archives of*

*Gynecology and Obstetrics*, 306(3), 663-686.

[7] **Baunoch, D., Luke, N., Wang, D., Vollstedt, A., Zhao, X., Ko, D. S., ... & Sirls, L. T. (2021).** Concordance between antibiotic resistance genes and susceptibility in symptomatic urinary tract infections. *Infection and drug resistance*, 3275-3286.

[8] **Soares, A., Alexandre, K., & Etienne, M. (2020).** Tolerance and persistence of *Pseudomonas aeruginosa* in biofilms exposed to antibiotics: Molecular mechanisms, antibiotic strategies and therapeutic perspectives. *Frontiers in microbiology*, 11, 2057.

[9] **Amer, M., & Awwad, A. (2020).** Green synthesis of copper nanoparticles by Citrus limon fruits extract, characterization and antibacterial activity.

[10] **Wojnicz, D., Kucharska, A. Z., Sokół-Łętowska, A., Kicia, M., & Tichaczek-Goska, D. (2012).** Medicinal plants extracts affect virulence factors expression and biofilm formation by the uropathogenic *Escherichia coli*. *Urological research*, 40(6), 683-

[11] **Coenye, T. (2023).** Biofilm antimicrobial susceptibility testing: where are we and where could we be going. *Clinical microbiology reviews*, 36(4), e00024-23.

[12] **Fu, Y., Yang, J. C., Cunningham, A. B., Towns, A. M., Zhang, Y., Yang, H. Y., ... & Yang, X. F. (2018).** A billion cups: The diversity, traditional uses, safety issues and potential of Chineseherbal teas. *Journal of ethnopharmacology*, 222, 217-228.

[13] **Maggini, V., Semenzato, G., Gallo, E., Nunziata, A., Fani, R., & Firenzuoli, F. (2024).** Antimicrobial activity of *Syzygium aromaticum* essential oil in human health treatment. *Molecules*, 29(5), 999.

[14] **Liu, J., Mahmood, M. S., Abbas, R. Z., Dillawar, A., Nawaz, Z., Luqman, M., ... & Rafique, A. (2021).** Therapeutic

appraisal of ethanolic and aqueous extracts of clove (*Syzygium aromaticum*) and garlic (*Allium sativum*) as antimicrobial agent. *Pakistan Journal of Agricultural Sciences*, 58(1).

[15] **Harborne, J. B. (1984).** Phenolic compounds. In *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (pp. 37-99). Dordrecht: Springer Netherlands

[16] **Perez, L.; Pauli, M. and Bazequire, P (1990)** Antibiotic assay by the agar well diffusion method .*Journal of Actabiology*. 15: 113 –115.

[17] **Salman, H. S., Majeed, H. M., & Shallal, Y. F. (2026).** Prevalence, Etiology, and Antibiotic Resistance Patterns of Urinary Tract Infections in a Baghdad Hospital: Focus on Uropathogenic *E. coli* and Virulence Factors. *International Journal of Microbiology*, 2026(1), 9117996.

[18] **Larkin, C., Valappil, S. P., & Palanisamy, N. (2025).** Global prevalence of nitrofurantoin-resistant uropathogenic *Escherichia coli* (UPEC) in humans: a systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy*, 80(10), 2609-2621.

[19] **Wadi, M. A., et al. (2025).** Evaluation of antibacterial activity and chemical analysis of clove aqueous extract. *BMC Complementary Medicine and Therapies*.