BACTERICIDAL ACTIVITIES OF SELECTED MEDICINAL PLANTS AGAINST MULTI-DRUG RESISTANT UROPATHOGENIC *ESCHERICHIA COLI* STRAINS

FARAH, N.[1] – BUGHLANI, M. S.[1] – RIAZ, A.[1] – ZAHRA, N.[1] – SARWAR, A.[2] – KHAN, A. A.[3] – AZIZ, T.[4*] – ALHARBI, M.[5] – ALBEKAIRI, T. H.[5] – ALASMARI, A. F.[5]

[1]*Institute of Molecular Biology & Biotechnology, University of Lahore, Lahore, Pakistan*

[2]*Food and Biotechnology Research Lab, PCSIR Complex, Lahore, Pakistan*

[3]*Department of Biotechnology, University of Malakand, 18800 Chakdara, Dir (Lower), Khyber Pakhtunkhwa, Pakistan*

[4]*Laboratory of Animal Health Food Hygiene and Quality, University of Ioannina, 47100 Arta, Greece*

[5]*Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, P.O. Box 2455, 11451 Riyadh, Saudi Arabia*

[*]*Corresponding author*
e-mail: iwockd@gmail.com

(Received 13th Sep 2024; accepted 18th Dec 2024)

Abstract. Pathogenic microbes, including *Bacillus thuringiensis*, *Staphylococcus*, and *Escherichia coli*, commonly infect the urogenital tract, often leading to urinary tract infections (UTIs) in human. Due to bacterial resistance to antibiotics and their side effects have prompted researchers to seek natural alternatives. Medicinal herbs are the promising alternatives to antibiotics and synthetic drugs. Medicinal herbs, including *Allium sativum* (garlic), *Zingiber officinale* (ginger), and *Nigella sativa* (kalonji), are increasingly recognized in scientific research for their potent antimicrobial properties and therapeutic potential. This study evaluated the antibacterial potential of garlic, ginger, and kalonji extracts against UTI-causing pathogens. Fresh samples of these herbs were obtained from local markets in Lahore, and their extracts were prepared using ethanol, n-hexane, and aqueous methods. Antimicrobial activity was assessed using the disc diffusion method, measuring inhibition of zones for bacterial biofilm formation, adherence, and motility. Results indicated that ethanol extracts of garlic and ginger exhibited the highest antibacterial activity, with inhibition zones of 10–11 mm against all tested pathogens. n-Hexane extracts showed moderate activity, while aqueous extracts were largely ineffective. Kalonji extracts demonstrated lower antimicrobial efficacy compared to garlic and ginger. The findings suggest that ethanol and n-hexane extracts of garlic and ginger are promising natural alternatives for treating UTIs caused by drug-resistant bacteria.
Keywords: *pathogens, antimicrobial, ethanol extracts, urinary tract infections*

Introduction

Urinary tract infections (UTIs) is a significant health issue (Dhanasekaran, 2019; De Miguel et al., 2020). Approximately 150 million individuals are affected by UTIs annually, primarily due to prevalent bacterial pathogens. In the elderly, UTIs are a leading cause of morbidity and mortality, accounting for 16% of hospitalizations and 6.5% of deaths (Cassini et al., 2016; Zalewska-Piątek and Piątek, 2020). UTIs are more prevalent in females than males, due to anatomical differences, behavioral factors, and the use of spermicides and diaphragms (Flores-Mireles et al., 2015). These infections can affect women at any age, with higher incidence rates associated with sexual activity, pregnancy, infancy in male infants, and advanced age in men (Foxman, 2010).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

In 80% of cases, the most common cause of catheter-associated infections or uncomplicated UTIs is *Escherichia coli*. In UTIs, other common bacteria that are involved are *Enterobacter, Enterococci, Staphylococcus saprophyticus, Staphylococcus aureus, Klebsiella pneumoniae,* and *Proteus species* (Kline and Bowdish, 2016). *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are rare casual factors of UTIs but they are quite important during Multi-Drug Resistance (Grygorcewicz et al., 2021).

Excessive antibiotic use and prophylactic treatments for UTIs have led to the emergence of multidrug-resistant bacteria. Because of this, it is very difficult to treat UTIs (Pires et al., 2017). They become resistant to antibiotics or the host immune system due to multiple or single-species biofilm. In recent years, the use of antibiotics has been restricted due to the high prevalence of MDR bacterial species (Chegini et al., 2021).

In the last few years, researchers have been trying to control infectious and pathogenic diseases by using commercially available antimicrobial drugs. The main hindrance in controlling microbial pathogenicity and infectious diseases is drug resistance (Fu et al., 2007). Drug-resistant bacterial strains are highly prevalent and a major threat to public health (Tängdén and Giske, 2015). To combat these issues, various therapies are used for treatment. But in various countries, medicinal herbs are used for the treatment of diseases because they are safe and cost-effective as compared to synthetic medicines, which result in severe side effects (Gupta et al., 2016).

Medicinal plants, such as *Allium sativum* (desi garlic), *Zingiber officinale* (white ginger), and *Nigella sativa* (mature seed kalonji), have been extensively documented for their antimicrobial, anti-inflammatory, and antioxidant properties in various scientific studies (Bouchama et al., 2024; Zawar et al., 2023; Naveed et al., 2023; Gambogou et al., 2018). Medicinal plants are the source of various bioactive compounds that are used for the treatment of various diseases (Hafiz et al., 2025; Zahra et al., 2024; Khadam et al., 2024; Ahmad et al., 2023a; Ahmad et al., 2023b; Hussain et al., 2023; Khurshaid et al., 2023; Bashir et al., 2023). They have therapeutic and pharmacological properties due to various phytochemical compounds like sesquiterpenes, diterpenes, monoterpenes, terpenoids, tannins, flavonoids, steroids, saponins, alkaloids, glycosides, and many others (Naveed et al., 2025; Aziz et al., 2024; Gul et al., 2023; El-Saber Batiha et al., 2020). But towards this aspect, a lot of research work is required because only a limited number of medicinal plants are known. Through ethnobotanical surveys, medicinal plants can be searched out (Marasini et al., 2015). In this study, the antimicrobial properties of three indigenous plants, including *Allium sativum*, *Zingiber officinale*, and *Nigella Sativa* were analyzed by making their n-hexane, ethanol, and aqueous extracts against local uropathogenic species of *Bacillus thruingiensus*, *Staphylococcus* and *Escherichia coli*.

Allium sativum (Garlic) has antibiotic resistance against several bacteria such as *E. coli, Salmonella, Shigella Sooner, Pseudomonas,* etc. Garlic is an ethnopharmaceutical drug but is also proved to have therapeutic effects by different scientific studies Allicin is an organosulfur compound, which prevents lipids from biosynthesis. It was proved in a study that it damages the Candida albicans cell wall and inhibits RNA in bacteria. Pure allicin has been found effective against isolates of Aspergillus in vitro study (Zhou and Wang, 2009). Bioactive compounds of allium species are used for treating cancer, aging, fungal, microbial diseases, immune system problems, infectious diseases, cardiovascular diseases, and many others.

Zingiber officinale (Ginger) belongs to the Zingiberaceae family. It is used as a medicinal plant for fever, wounds, indigestion, hypertension, vomiting, constipation, pains, sore throats, sprains, rheumatism, and treatment of cramps (Ali et al., 2008).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

Literature studies have shown that ginger has antimicrobial activities, like anticoagulant, anti-inflammatory, antifungal, and antibacterial (Nurtjahja-Tjendraputra et al., 2003; Singh et al., 2005; Kumar et al., 2013). Ginger oil contains several compounds like lipophilic compounds that made the cytoplasmic membrane and cell wall more permeable, as a result integrity of the membrane is lost in fungi (Moon et al., 2018).

Nigella sativa (kalonji) is a herbaceous plant that has great medicinal value and has been found to exhibit various medicinal properties like anti-inflammatory, antioxidant, antiviral, antifungal, antibacterial, and anti-parasitic (Ali et al., 2008). Various studies have indicated the presence of bioactive compounds like t-anethole benzene, longifolene, 4-terpineol, alpha-pinene, p-cymene, carvacrol, limonene, thymol, and thymoquinone in its seeds (Toma et al., 2010). This plant seeds have pyrazole alkaloids like nigericin and nigellone and isoquinoline alkaloids like nigellone-N-oxide (Kooti et al., 2016). studies have shown that extracts of N. sativa have synergistic effects in inhibiting the growth of *E. coli* along with cephalexin, terbinafine, nalidixic acid, ampicillin, chloramphenicol, doxycycline, streptomycin, and gentamicin (Sadri et al., 2013).

Considering the importance of herbal products as treatments for infectious diseases, the antimicrobial activity of aqueous and ethanolic extracts of the three mentioned plant species from Lahore, Pakistan on MDR *Bacillus thruingiensus*, *Staphylococcus* and *Escherichia coli*. isolates from patients suffering from urinary tract infection (UTI) were examined in the current study.

The *Staphylococcus* species used in the study were identified at the species level through appropriate identification methods, such as biochemical testing, molecular techniques r16S rRNA gene sequencing. This ensured accurate identification of the specific *Staphylococcus* species responsible for urinary tract infections and allowed for a more precise evaluation of the antimicrobial effects of the plant extracts.

The selection of garlic, ginger, and Kalonji for this study is justified by their long-established antimicrobial properties, which have been widely recognized in both traditional and modern medicine. While their antibacterial effects have been confirmed over the years, the focus of this research is to evaluate their effectiveness against specific uropathogenic strains, particularly in light of the increasing prevalence of antibiotic resistance. Additionally, exploring the antibacterial potential of these commonly used plants in contemporary contexts, such as biofilm formation, adherence, and motility of uropathogenic bacteria, provides valuable insights into their potential as natural alternatives to synthetic antibiotics.

The objective of this research is to evaluate the antimicrobial properties of garlic (*Allium sativum*), ginger (*Zingiber officinale*) and Kalongi (*Nigella sativa*) extracts on local uropathogenic species of *Bacillus thruingiensus*, *Staphylococcus* and *Escherichia coli* that are involved in most of the cases of urinary tract infections (UTI's).

Materials and methods

Sample collection

Zingiber officinale (ginger), *Allium sativum* (garlic), and *Nigella sativa* (kalonji), which were employed in the current study, were all obtained from Lahore's local market in Pakistan. To prevent microbial contamination, dried slices of ginger, garlic, and kalonji were each crushed to a powder by using a blender before being kept for further processing in a plastic bag.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

Plant materials and extraction procedures

In the case of garlic (*Allium sativum*), the bulbs were likely utilized, as they are the primary part of the plant known for their antimicrobial properties. Similarly, for ginger (*Zingiber officinale*), the rhizomes (underground stems) are typically used for extraction. As for Kalonji (*Nigella sativa*), the seeds are the most commonly used part for medicinal purposes.

S. aureus uses synthetic antibiotic or drug with established antimicrobial activity (e.g., amoxicillin, ciprofloxacin). Dimethyl sulfoxide (DMSO) was used as a negative control because this aprotic polar solvent has capability to dissolve polyeugenol and eugenol compounds as test samples and is a solvent that has no antibacterial properties.

The autoclave was used to sterilize nine beakers for 15 minutes at 121°C. Separately, 150 ml of distilled water, ethanol, and n-hexane were soaked with 50 g powder each of the kalonji, ginger, and garlic pastes. The flasks were shaken at 120 rpm while being incubated for 72 hours at room temperature. At 25°C, the crude extracts were centrifuged for 15 minutes at 2000 rpm. On a rotary evaporator, the aqueous extracts were evaporated at 80°C while the n-hexane and ethanol extracts were done so at 50°C. To eliminate the remaining undissolved materials, all dry extract samples were centrifuged one more at 10,000 rpm after being individually diluted in distilled water to a final concentration of 100 mg/ml. At 4°C, the extract solutions were kept. The controls, n-hexane, ethanol, and water, were likewise handled similarly to how to extract extraction was described. In each extract, a few drops of DMSO (Dimethyl sulfoxide) were added and stored for further testing.

Isolation and identification of bacterial strains

The detected and isolated colonies were streaked on the nutrient agar plates to purify the bacteria. The inoculums were uniformly subcultured on other selective and differential media Mac using a sterile microbiology loop. Pseudomonas Cetrimide Agar (PCA), Conkey Agar, Blood Agar, and Polymyxin Pyruvate Egg Yolk Eosine Methylene Blue (EMB), *Salmonella* Shigella agar (SS-agar), Mannitol Bromothymol Blue Agar Base (PEMBA), and Mannitol Salt agar (MSA). Using spore staining and gram staining, the microscopic appearance and organization of pure bacteria were investigated (Devi et al., 2022).

Biochemical tests of identified bacterial strains

Many biochemical tests, including the catalase, oxidase, and coagulase tests. According to previously described protocols, tests such as casein hydrolysis, lipid, starch, triple sugar iron (TSI), hydrogen sulfide test (H2S), urease test, citrate utilization test, Voges-Proskauer test, methyl red test, indole production test were carried out to confirm isolated bacterial cultures on a species level (Devi et al., 2022).

Antimicrobial activity

The disc diffusion technique was used to perform the antibacterial activity of extract of each selected plants against each selected spices separately as reported by Kirby-Bauer (1996). Every experiment was carried out under sterile surroundings. Each test bacterial strain culture was put onto a separate nutrient agar plate with 107 CFU, and each plate's surface was covered uniformly. The sterile discs (5 mm in diameter) were put atop nutrient agar plates seeded with bacterial culture after being aseptically submerged in

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

various extracts for one minute. After 15 minutes at room temperature, the plates were incubated at 37°C for 16 hours, and the presence of an inhibitory zone was checked. Inhibition zones' diameters were measured in millimeters. Each bacterial strain was subjected to a triple antimicrobial test.

Results

Antimicrobial assay results

The antimicrobial effect of ethanol, n-hexane and aqueous extracts of garlic, ginger and kalonji against *E. coli, Staphylococcus aureus* and *Bacillus thuringiensis* was evaluated by disc diffusion method showed in tables.

Antimicrobial activity of garlic

Garlic antimicrobial activity against *E. coli* showed by produced zones of inhibition of 10 mm by ethanolic extract, 8 mm by distilled water and 7 mm by n-hexane extract, against *Staphylococcus aureus* 11 mm by ethanolic extract, 7.5 mm by distilled water and 0 mm by n-hexane extract and against *Bacillus thuringiensis* 10 mm by ethanolic extract, 6 mm by distilled water and 9 mm by n-hexane extract that showed in *Table 1, Figure 1*.

Table 1. Antibacterial activity of n-hexane, ethanolic, and distilled water extract of garlic against *E. coli, S. aureus, B. thuringiensis*

Antibacterial activity of garlic	***E. coli***	***S. aureus***	***B. thuringiensis***
Extract	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
n-hexane extract	7	0	9
Ethanolic extract	10	11	10
Distilled water	8	7.5	6

Figure 1. *The anti-bacterial activity of E. coli, S. aureus, B. thuringiensis against the n-hexane, ethanolic, and distilled water extract of garlic*

Antimicrobial activity of ginger

Ginger antimicrobial activity against *E. coli* showed by produced zones of inhibition of 15 mm by ethanolic extract, 0 mm by distilled water and 10.5 mm by n-hexane extract, against *Staphylococcus aureus* 17 mm by ethanolic extract, 9 mm by distilled water and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

0mm by n-hexane extract and against *Bacillus thuringiensis* 15.5 mm by ethanolic extract, 0mm by distilled water and 0mm by n-hexane extract that showed in *Table 2, Figure 2*.

Table 2. Antibacterial activity of n-hexane, ethanolic, and distilled water extract of ginger against *E. coli, S. aureus, B. thuringiensis*

Antibacterial activity of Ginger	***E. coli***	***S. aureus***	***B. thuringiensis***
Extract	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
n-hexane extract	10.5	0	0
Ethanolic extract	15	17	15.5
Distilled water	0	9	0

(i) (ii) (iii)

Figure 2. *The anti-bacterial activity of E. coli, S. aureus & B. thuringiensis against the n-hexane, ethanolic, and distilled water extract of ginger*

Antimicrobial activity of kalonji

Kalonji antimicrobial activity against *E. coli* showed by produced zones of inhibition of 7 mm by ethanolic extract, 0 mm by distilled water and 5 mm by n-hexane extract, against *Staphylococcus aureus* 0.5 mm by ethanolic extract, 0 mm by distilled water and 3 mm by n-hexane extract and against *Bacillus thuringiensis* 0 mm by ethanolic extract, 0 mm by distilled water and 4 mm by n-hexane extract that showed in *Table 3, Figure 3*.

Table 3. *Antibacterial activity of n-hexane, ethanolic, and distilled water extract of kalonji against E. coli, S. aureus, B. thuringiensis*

Antibacterial activity of Kalonji	***E. coli***	***S. aureus***	***B. thuringiensis***
Extract	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
n-hexane extract	5	3	4
Ethanolic extract	7	0.5	0
Distilled water	0	0	0

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

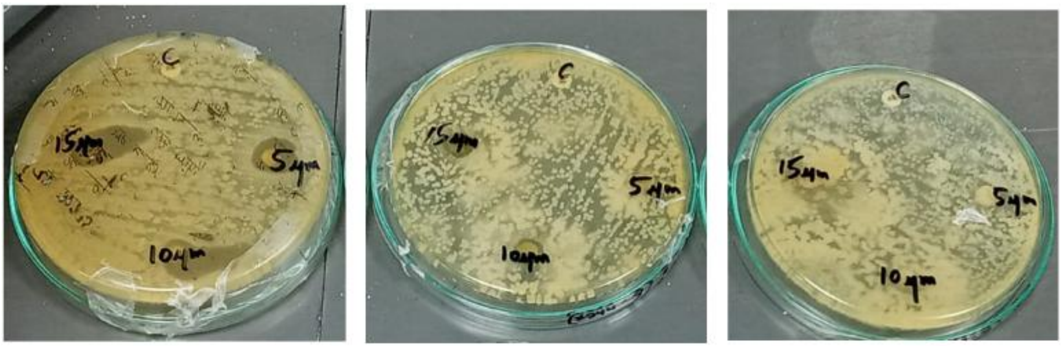

Figure 3. *The anti-bacterial activity of E. coli, S. aureus & B. thuringiensis against the n-hexane, ethanolic, and distilled water extract of kalonji*

Discussion

Urinary tract infections (UTIs), results in approximately 400 million cases and billions of dollars are spend every year in healthcare sector. While uropathogenic *Escherichia coli* is the most common cause, other pathogens like *Klebsiella*, *Enterococcus*, *Pseudomonas*, *Staphylococcus,* and *Candida* species can also lead to UTIs (Timm et al., 2024). Urinary tract infections: pathogenesis, host susceptibility and emerging therapeutics. The current study was designed to investigate the antimicrobial activity of extracts from native medicinal plants in n-hexane, ethanol and distilled water. These extracts included extracts of garlic, ginger, and kalonji in various solvents against strains of uropathogenic multidrug-resistant bacteria, such as *E. coli*, *S. aureus,* and *B. thruingiensus.* Antimicrobial activity was analyzed by measuring zone of inhibition using disc diffusion method. The study focuses on evaluating the effectiveness of the plant extracts against resistant bacterial strains, without performing statistical processing or making direct comparisons between treatments.

In this study, the antimicrobial activity was found to be highest in ginger, followed by garlic and Kalonji against Escherichia coli, *S. aureus and B. thruingiensus*. *Our* findings showed that various spice extracts have a wide range of antibacterial activity, and the examined bacterial species' sensitivity to these extracts varied. Garlic extract exhibits antimicrobial activity against *E. coli*, *S. aureus*, and *B. thuringiensis,* with the ethanolic extract showing the strongest inhibition. The zones of inhibition were 10mm for *E. coli*, 11 mm S. *aureus,* and 10 mm for *B. thuringiensis*. However, distilled water and n-hexane extracts showed less inhibition as compared to ethanolic extract. The antimicrobial activity of Garlic is primarily due to allicin, an active ingredient of garlic that targets a broad range of microorganisms, including antibiotic-resistant, Gram-positive and Gram-negative bacteria like *Shigella*, *E. coli*, *S. aureus*, *P. aeruginosa* etc. (El-Saber Batiha et al., 2020).

Data showed that n-Hexane, ethanol and distilled water extracts of ginger showed antimicrobial activity against multidrug resistance *E. coli* as 10.5 mm, 15 mm and 0 mm, respectively. Among three extracts ethanolic extract of ginger showed pronounced inhibitory activity against *E. coli*. Our findings are in accordance with Nader et al. (2010) who revealed ethanolic extract of ginger was more effective against *E. coli.*

Ginger inhibitory activity against *S. aureus* revealed that ethanolic extract was more effective then n-hexane and distilled water extract. Ethanolic extract exhibited 17 mm diameter of zone of inhibition. against *S. aureus*. However, n-Hexan ginger extract

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

showed no inhibition against *S. aureus*. This inhibitory activity is due to constituent of Ginger, particularly gingerol and shagelol that have been reported to demonstrate antimicrobial properties against bacterial strains like *E. coli*, *Salmonella typhi*, and *Candida albicans*, as well as antifungal effects (Rahmani and Aly, 2014).

Ginger extract in different solvent revealed antimicrobial activity against *Bacillus thuringiensis* 15.5 mm by ethanolic extract. However, *Bacillus thuringiensis* become resistant against distilled water and n-hexane extract with no zone of inhibition. Our findings are in agreement with Pillai et al. (2010) who reported ethanolic extract was effective against Bacillus species and aquaous extract of ginger was not effective against *B. thuringiensis.*

Results suggested that ethanolic extract of garlic was found to be most efficient against target species as compared to other extracts. Garlic and ginger ethanolic and n-hexane extracts showed antibacterial potential and should be preferred for medicinal purposes.

Kalonji exhibits antimicrobial activity against *E. coli*, *S. aureus,* and *B. thuringiensis,* with the ethanolic extract showing the strongest inhibition: 7 mm for *E. coli,* 0.5 mm for *S. aureus,* and no activity against *B. thuringiensis*. The n-hexane extract showed moderate activity, with inhibition zones of 5 mm, 3 mm, and 4 mm, respectively. However, distilled water extract is not effective against any pathogen. Our findings are in accordance with the previous reports in which ethanolic extract showed more pronounced inhibitory activity and aqueous extract was least effective against different pathogens (Nasir and Irshad, 2015).

Kalonj (*N. sativa*) and its bioactive compound, thymoquinone, induce oxidative stress, cell apoptosis, and increase membrane permeability. They also inhibit efflux pumps and exhibit strong biocidal effects (Hossain et al., 2021).

Among all three spices, distilled water extract showed minimum zone of inhibition which shows that it should not be preferred for medicinal purposes, some other extracts might be preferred like methanol in future studies. These results are supported by Atsamnia and coworkers predicted the zone of inhibition of garlic extracts by using artificial neural network method. F3Results suggested that as compared to time and temperature most determinant variable is concentration of extract, which means higher the concentration greater will be the zone of inhibition (Atsamnia et al., 2017). One of the most commonly used herb for medicinal purposes is garlic. Wolfe and coworkers conducted a research study for examining the antibacterial activities of garlic extracts against some selected bacterial species including: *E. coli* and *S. aureus*. In this research study, chloroform garlic extract showed greater zone of inhibition. *S. aureus* was found to be less susceptible to petroleum, chloroform and ethanol extracts as compared to *E. coli* extracts. So, they suggested that it can used as an antibacterial agent (Ochieng Nyalo et al., 2022). To evaluate the antibacterial efficacy of methanol and aqueous garlic extracts against six uropathogenic species: *Serratia marcescens*, *Klebsiella spp.*, *Proteus spp.*, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, and *Escherichia coli*, with *E. coli* serving as the reference strain. The findings demonstrated that *Allium sativum* (garlic) extract exhibited antibacterial activity against the tested uropathogens. However, the study highlighted the need to purify and isolate the active compounds from the plant extracts for potential use in drug or medicine development (Lionel et al., 2020). The antimicrobial activity of ethanolic *Allium sativum* extract against bacterial strains isolated from urinary tract infections. The study revealed that these bacterial strains exhibited significant resistance to the antibiotic Ceftazidime. However, *Allium sativum* extract demonstrated potent antibacterial activity against key uropathogenic species, including

Proteus mirabilis, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*, which are known to cause urinary tract infections (Moustafa et al., 2020). The potential of *Aloe vera*, *Kalonji*, and *Miswak* extracts as effective antibacterial agents against pathogens associated with UTIs, particularly when used in combination. The study also highlighted their promising antioxidant and anti-proliferative activities, suggesting their potential application in managing UTIs and other related conditions. Superoxide dismutase assays and Catalase and were used to assess anti-proliferative activity and antioxidant activity. Plant extracts, both alone and in combination, were proven to be highly efficient antibacterial agents against standard strains of *Candida albicans* and *Staphylococcus aureus* (Amjed et al., 2017). The *N. sativa* seeds antimicrobial activity of different extracts of extracted thymoquinone, and oil samples against *Bacillus licheniformis* and *Bacillus subtilis* was studied in this work. Although methanol extract contained the most phytochemicals, hexane extract was the most powerful in regards of antibacterial action. Both of these solvents were used to extract thymoquinone which is a therapeutic significant chemical in *N. sativa* seed (Habib and Choudhry, 2021).

Alcohol extracts of *Zingiber officinale* (ginger) and *Allium sativum* (garlic) exhibited significant antibacterial activity against various pathogenic bacterial strains, including *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Escherichia coli*. This study employed plant powder extracted using 70% alcohol, followed by dilution and well-diffusion methods to evaluate the extracts. Consistent with our findings, their results confirmed that all tested bacterial species were susceptible to the extracts, underscoring the potential of these plants as natural antimicrobial agents for managing infections caused by multidrug-resistant bacteria (Benyagoub et al., 2021). The antibacterial activities observed in various extracts of selected spices can be attributed to their phytochemical constituents. In garlic, alliin is recognized as a key compound with potent antibacterial properties. Allicin, a toxic compound derived from alliin, acts against bacterial species by inhibiting DNA, RNA, and protein synthesis, with RNA synthesis being its primary target. Diallylthiosulfinate, commonly known as allicin, functions as a defense molecule and demonstrates significant antimicrobial activity even at minimal concentrations of the extract. It interacts with thiol groups and enhances the activity of enzymes that play a role in combating diseases. Similarly, ginger contains important phytochemicals such as phenolics, sesquiterpenoids, and monoterpenoids, along with their derivatives, including esters, alcohols, ketones, and aldehydes, which exhibit antimicrobial properties. It is particularly rich in bioactive compounds like terpenes and phenolics, as well as their derivatives such as paradols, shogaols, and gingerols, known for their antimicrobial effectiveness. In *Kalonji* (black seed), essential oils such as arborvitae, clary sage, lavender, clove, thyme, and oregano contribute to its antimicrobial properties. These findings underscore the role of plant-derived phytochemical compounds in disease treatment, highlighting the growing interest of traditional practitioners in natural medicines.

Conclusion

Results of research study showed therapeutic efficacy of garlic, ginger and kalonji ethanol, n-hexane and aqueous extracts with detailed explanation. Selected plants showed antibacterial properties against most common uropathogens (*Bacillus thruingiensus*, *Staphylococcus* and *Escherichia coli*). There are lot of benefits of using plant spices and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

their phytochemical compounds as an alternate to antibiotics that reduce the chance of developing antibiotic-resistant microbes which results from frequent use of antibiotics (abuse, misuse) and reducing the cost of purchasing synthetic medicines. Present research study justifies the antibacterial potential of selected plants extracts against uropathogens. So, in future there is a need to isolate and purify bioactive compounds from plant extracts that could be used as a source of antimicrobial drugs. In future studies, researcher must carry out in vivo studies for examining the antimicrobial efficacy of medicinal herbs against different kinds of microbial species. So, that population of developing countries can obtain low-cost treatment for prevention of life-threatening infections.

Conflicts of interest. The authors declare that they have no conflict of interest.

Acknowledgments. The authors greatly acknowledge and express their gratitude to the Researchers Supporting Project number (RSP2025R335), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

[1] Ahmad, E., Jahangeer, M., Mahmood Akhtar, Z., Aziz, T., Alharbi, M., Alshammari, A., Alasmari, A. F., Irfan Bukhari, N. (2023a). Characterization and gastroprotective effects of Rosa brunonii Lindl. fruit on gastric mucosal injury in experimental rats - A preliminary study. – Acta Biochim Pol. 70(3):633-641.

[2] Ahmad, E., Jahangeer, M., Irfan Bukhari, N., Sarwar, A., Aziz, T., Alharbi, M., Alshammari, A., Alasmari, A. F. (2023b): Isolation, Structure Elucidation & Antidiabetic Potential of *Rosa brunonii* L. fruit – Fight Diabetes with Natural Remedies. – J. Chil. Chem. Soc. 68(2): 5887-5894.

[3] Ali, B. H., Blunden, G., Tanira, M. O., Nemmar, A. (2008): Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. – Food and chemical Toxicology 46(2): 409-420.

[4] Amjed, S., Junaid, K., Jafar, J., Amjad, T., Maqsood, W., Mukhtar, N., Tariq, K., Sharif, M., Awan, S. J., Ansari, F. (2017): Detection of antibacterial activities of Miswak, Kalonji and Aloe vera against oral pathogens & anti-proliferative activity against cancer cell line. – BMC Complementary and Alternative Medicine 17(1): 1-10.

[5] Atsamnia, D., Hamadache, M., Hanini, S., Benkortbi, O., Oukrif, D. (2017): Prediction of the antibacterial activity of garlic extract on E. coli, S. aureus and B. subtilis by determining the diameter of the inhibition zones using artificial neural networks. – LWT-Food Science and Technology 82: 287-295.

[6] Aziz, T., Qadir, R., Anwar, F., Naz, S., Nazir, N., Nabi, G., Haiying, C., Lin, L., Alharbi, M., Alasmari, A. F. (2024). Optimal Enzyme-Assisted Extraction of Phenolics from Leaves of Pongamia pinnata via Response Surface Methodology and Artificial Neural Networking. – Appl Biochem Biotechnol. 196(9):6508-6525.

[7] Bashir, A., Muhammad Yousafzai, A., Maria, H., Khan, A. A., Aziz, T., Alharbi, M., Alsahammari, A., Alasmari, A. F. (2023). Curative Effects of Dianthus orientalis against Paracetamol Triggered Oxidative Stress, Hepatic and Renal Injuries in Rabbit as an Experimental Model. – *Separations 10*: 182.

[8] Benyagoub, E., Nabbou, N., Aguid, A., Alkhudhairy, M. K., Bendada, F. (2021): In vitro antibacterial activity of fenugreek seeds’ phytoconstituents from Taghit Region (Southwest of Algeria) against the bacterial strains responsible for UTI. – Current Bioactive Compounds 17(4): 339-355.

[9] Bouchama, C., Yousfi, L. K., El Akhdari, S., Chadli, N., Samouh, K. F., Raoui, S. M., Aziz, T., Zinedine, A., Errachidi, F. (2024): Effect of feed supplementation with Curcuma longa

and Zingiber officinale spices on the zootechnical, biochemical, and microbiological parameters of broiler chickens. – Notulae Scientia Biologicae 16(3): 11948.
[10] Cassini, A., Plachouras, D., Eckmanns, T., Abu Sin, M., Blank, H.-P., Ducomble, T., Haller, S., Harder, T., Klingeberg, A., Sixtensson, M. (2016): Burden of six healthcare-associated infections on European population health: estimating incidence-based disability-adjusted life years through a population prevalence-based modelling study. – PLoS medicine 13(10): 100-150.
[11] Chegini, Z., Khoshbayan, A., Vesal, S., Moradabadi, A., Hashemi, A., Shariati, A. (2021): Bacteriophage therapy for inhibition of multi drug-resistant uropathogenic bacteria: a narrative review. – Annals of Clinical Microbiology and Antimicrobials 20(1): 1-13.
[12] De Miguel, T., Rama, J. L. R., Sieiro, C., Sánchez, S., Villa, T. G. (2020): Bacteriophages and lysins as possible alternatives to treat antibiotic-resistant urinary tract infections. – Antibiotics 9(8): 466.
[13] Devi, S. A., Devaki, K., Rajasri, M., Jyothsna, M. (2022): Collection and isolation of Bacillus thuringiensis Berliner from the soils of cruciferous vegetable growing areas in Andhra Pradesh. – The Pharma Innovation International Journal 11(2).
[14] Dhanasekaran, S. (2019): Research article alteration of multi-drug resistance activities by ethanolic extracts of Nigella sativa against urinary pathogens. – Int J Pharmacol 15(8): 962-969.
[15] El-Saber Batiha, G., Magdy Beshbishy, A., Stephen Adeyemi, O., Nadwa, E., Rashwan, E., Yokoyama, N., Igarashi, I. (2020): Safety and efficacy of hydroxyurea and eflornithine against most blood parasites Babesia and Theileria. – PloS One 15(2): 0228996.
[16] El-Saber Batiha, G., Beshbishy, A. M., Wasef, L. G., Elewa, Y. H. A., Al-Sagan, A. A., Abd El-Hack, M. E., Prasad Devkota, H. (2020): Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. – Nutrients 12(3): 872.
[17] Flores-Mireles, A. L., Walker, J. N., Caparon, M., Hultgren, S. J. (2015): Urinary tract infections: epidemiology, mechanisms of infection and treatment options. – Nature reviews Microbiology 13(5): 269-284.
[18] Foxman, B. (2010): The epidemiology of urinary tract infection. – Nature Reviews Urology 7(12): 653-660.
[19] Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S., Efferth, T. (2007): Antimicrobial activity of clove and rosemary essential oils alone and in combination. – Phytotherapy Research 21(10): 989-994.
[20] Gambogou, B., Ouattara, A. K., Taale, E., Karou, S. D., Ameyapoh, Y. A., Simpore, J. (2018): Garlic as alternative therapy to treat uropathogene bacteria in women with urinary tract infection in Lomé, Togo. – European Journal Pharmaceutical and Medical Research 5(12): 1-7.
[21] Grygorcewicz, B., Wojciuk, B., Roszak, M., Łubowska, N., Błażejczak, P., Jursa-Kulesza, J., Rakoczy, R., Masiuk, H., Dołęgowska, B. (2021): Environmental phage-based cocktail and antibiotic combination effects on Acinetobacter baumannii biofilm in a human urine model. – Microbial Drug Resistance 27(1): 25-35.
[22] Gupta, D., Dubey, J., Kumar, M. (2016): Phytochemical analysis and antimicrobial activity of some medicinal plants against selected common human pathogenic microorganisms. – Asian Pacific Journal of Tropical Disease 6(1): 15-20.
[23] Gul, R., Rahmatullah, Q., Ali, H., Bashir, A., Ayaz, A. K., Tariq, A., Metab, A., Abdulrahman, A., Abdullah, F. A. (2023): Phytochemical, Anitmicrobial, Radical Scavening and In-vitro biological activities of Teucurium stocksianum leaves. – J. Chil. Chem. Soc. 68 (1): 5748-5754.
[24] Habib, N., Choudhry, S. (2021): HPLC quantification of thymoquinone extracted from *Nigella sativa* L. (Ranunculaceae) seeds and antibacterial activity of its extracts against Bacillus species. – Evidence-Based Complementary and Alternative Medicine, pp. 1-11.
[25] Rasheed, H. A., Rehman, A., Chen, X., Aziz, T., Al-Asmari, F., Alhomrani, M., Alamri, A. S., Cui, H., Lin, L. (2024). Unveiling the anti-listerial effect of Citrus bergamia essential

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

oil: Mechanism of membrane disruption and anti-hemolytic activity. – Food Bioscience 61 104742.
[26] Hossain, M. S., Sharfaraz, A., Dutta, A., Ahsan, A., Masud, M. A., Ahmed, I. A., Ming, L. C. (2021): A review of ethnobotany, phytochemistry, antimicrobial pharmacology and toxicology of *Nigella sativa* L. – Biomedicine & Pharmacotherapy 143: 112182.
[27] Hussain, Z., Jahangeer, M., Sarwar, A., Ullah, N., Aziz, T., Alharbi, M., Alshammari, A. (2023): Synthesis and Characterization of Silver Nanoparticles mediated by the Mentha piperita Leaves Extract and Exploration of its Antimicrobial Activities. – J. Chil Chem Soc. 68(2): 5865-5870.
[28] Khurshaid, I., Ilyas, S., Zahra, N., Ahmad, S., Aziz, T., Al-Asmari, F., Almowallad, S., Al-Massabi, R. F., Alanazi, Y. F., Barqawi, A. A., Tahir Kassim, R. M., Alamri, A. S., Alhomrani, M., Sameeh, M. Y. (2023): Isolation, preparation and investigation of leaf extracts of Aloe barbadensis for its remedial effects on tumor necrosis factor alpha (TNF-α) and interleukin (IL-6) by in vivo and in silico approaches in experimental rats. – Acta Biochim Pol. 70(4):927-933.
[29] Khadam, A., Alyas, S., Zahra, N., Ahmad, S., Sarwar, A., Khan, A. A., Aziz, T., Alharbi, M., Alasmari, A. F. (2024): Influence of *Azadirachta indica* leaf extracts on tumor necrosis factor-α and interleukin-6 in albino rats and its computational analysis. – Cell Mol Biol (Noisy-le-grand).;70(11):95-100.
[30] Kim, J., Kyung, K. (2003): Antiyeast activity of heated garlic in the absence of alliinase enzyme action. – Journal of Food Science 68(5): 1766-1770.
[31] Kirby-Bauer, A. (1996): Antimicrobial sensitivity testing by agar diffusion method. – J Clin Pathol 44: 493.
[32] Kline, K. A., Bowdish, D. M. E. (2016): Infection in an aging population. – Curr Opin Microbiol. 29: 63-67.
[33] Kooti, W., Hasanzadeh-Noohi, Z., Sharafi-Ahvazi, N., Asadi-Samani, M., Ashtary-Larky, D. (2016): Phytochemistry, pharmacology, and therapeutic uses of black seed (*Nigella sativa*). – Chinese journal of natural medicines 14(10): 732-745.
[34] Kumar, S., Saxena, K., Singh, U. N., Saxena, R. (2013): Anti-inflammatory action of ginger: A critical review in anemia of inflammation and its future aspects. – Int J Herb Med 1(4): 16-20.
[35] Lionel, O. O., Adegboyega, I. P., Ezekiel, A. O., Lufunke, B. C. (2020): Antimicrobial activity of garlic (*Allium sativum*) on selected uropathogens from cases of urinary tract infection. – Annals of Tropical Pathology 11(2): 133.
[36] Marasini, B. P., Baral, P., Aryal, P., Ghimire, K. R., Neupane, S., Dahal, N., Singh, A., Ghimire, L., Shrestha, K. (2015): Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. – BioMed Research International 2015: 265425.
[37] Moon, Y.-S., Lee, H.-S., Lee, S.-E. (2018): Inhibitory effects of three monoterpenes from ginger essential oil on growth and aflatoxin production of Aspergillus flavus and their gene regulation in aflatoxin biosynthesis. – Applied Biological Chemistry 61: 243-250.
[38] Moustafa, M., Mohamed, Z., Gheeth, D., Salah, M. (2019): Antimicrobial potential of various essential oils against multidrug-resistant uropathogenic *Escherichia coli*. – Int. J. Progress. Sci. Technol. 16: 198-203.
[39] Nader, M. I., Ghanima, K. K., Ali, S. A., Azhar, D. A. (2010): Antibacterial activity of ginger extracts and its essential oil on some of pathogenic bacteria. – Baghdad Science Journal 7(3): 1159-1165.
[40] Nasir, U., Irshad, S. (2015): Evaluation of the antimicrobial activity of aqueous, ethanolic and methanolic extracts of *Nigella sativa* (kalonji) against gram positive and gram negative bacterial isolates. – Pak. J. Biochem. Mol. Biol. 48(4): 92-96.
[41] Naveed, M., Ali, I., Aziz, T., Saleem, A., Rajpoot, Z., Khaleel, S., Khan, A. A., Alharbi, M., Albekairi, H. T. (2025): Computational and GC-MS screening of bioactive compounds

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423

from Thymus Vulgaris targeting mycolactone protein associated with Buruli ulcer. – Sci Rep. 15: 131

[42] Naveed, M., Makhdoom, S. I., Rehman, S. U., Aziz, T., Bashir, F., Ali, U., Alharbi, M., Alshammari, A., Alasmari, A. F. (2023): Biosynthesis and Mathematical Interpretation of Zero-Valent Iron NPs Using Nigella sativa Seed Tincture for Indemnification of Carcinogenic Metals Present in Industrial Effluents. – Molecules 28(8):3299.

[43] Nurtjahja-Tjendraputra, E., Ammit, A. J., Roufogalis, B. D., Tran, V. H., Duke, C. C. (2003): Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. – Thrombosis Research 111(4-5): 259-265.

[44] Ochieng Nyalo, P., Isanda Omwenga, G., Piero Ngugi, M. (2022): GC-MS analysis, antibacterial and antioxidant potential of ethyl acetate leaf extract of Senna singueana (delile) grown in Kenya. – Evidence-Based Complementary and Alternative Medicine 4(7): 34-45.

[45] Pillai, M. K., Asmawi, M. Z., Choon, T. S., Sasidharan, S., Lachumy, S. J., Ismail, R. (2010): Inhiibitory effect of ethanolic and water extract of two varities of ginger on selected bacterial and fungal isolates. – Nature 12(5): 67-78.

[46] Pires, D. P., Melo, L. D., Boas, D. V., Sillankorva, S., Zeredo, J. (2017): Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections. – Current Opinion in Microbiology 39: 48-56.

[47] Rahmani, A. H., Al Shabrmi, F. M., Aly, S. M. (2014): Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. – International Journal of Physiology, Pathophysiology and Pharmacology 6(2): 125-36.

[48] Sadri, S. M., Namjoo, A., Rafieian-kopaei, M., Ashrafi, K., Shahin Fard, N., Ansari Samani, R. (2013): Comparing the Effects of Nigella Sativa Extract and Gentamicin in Treatment of Urinary Tract Infection Caused by Ecoli. – J Mazandaran Univ Med Sci 22(96): 21-29.

[49] Singh, G., Maurya, S., Catalan, C., De Lampasona, M. (2005): Studies on essential oils, Part 42: chemical, antifungal, antioxidant and sprout suppressant studies on ginger essential oil and its oleoresin. – Flavour and Fragrance Journal 20(1): 1-6.

[50] Tängdén, T., Giske, C. (2015): Global dissemination of extensively drug-resistant carbapenemase-producing E nterobacteriaceae: clinical perspectives on detection, treatment and infection control. – Journal of Internal Medicine 277(5): 501-512.

[51] Toma, C.-C., Simu, G. M., Hanganu, D., Olah, N., Vata, F. M. G., Hammami, C., Hammami, M. (2010): Chemical composition of the Tunisian Nigella sativa. Note I. Profile on essential oil. – Farmacia 58(4): 458-464.

[52] Zalewska-Piątek, B., Piątek, R. (2020): Phage therapy as a novel strategy in the treatment of urinary tract infections caused by *E. coli*. – Antibiotics 9(6): 304.

[53] Zhou, J. Q., Wang, J. W. (2009): Immobilization of alliinase with a water soluble–insoluble reversible N-succinyl-chitosan for allicin production. – Enzyme and Microbial Technology 45(4): 299-304.

[54] Zahra, N., Mazhar, A., Zahid, B., Naeem, M. A., Sarwar, A., Aziz, T., Alharbi, M., Albekairi, T. H., Alasmari, A. F. (2024). Evaluation of influence of *Butea monosperma* floral extract on inflammatory biomarkers. – Open Chemistry 22(1): 20230199.

[55] Zawar, H., Muhammad, J., Shafiq, U. R., Tammana, I., Abid, S., Najeeb, U., Tariq, A., Metab, A., Abdulrahman, A. (2023): Synthesis of silver nanoparticles by aqueous extract of Zingiber officinale and their antibacterial activities against selected species. – Polish J. Chem. Tech. 25(3): 23-30.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24112423