RESEARCH ARTICLE

Evaluation of Antibacterial Activity of Aqueous and Alcohol Extracts of *Zingiber officinale* and *Allium sativum* on Bacterial Isolates from Urinary Tract Infection Patients

Ahmed K. Al-Kubeisi

Department of Medical Laboratory Techniques, Al-Nukhba University College, Baghdad, Iraq

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ABSTRACT

This study aimed to study the effect *Zingiber officinale* and *Allium sativum* crude extract on bacterial isolates from children suffer from urinary tract infection (UTI) and compare it with the activity ofantibiotics. The bacterial isolated were collected from variety of hospitals during the period 6-1-2021 to 5-4-2022. The isolates were cultured and identified. 100 bacterial isolates identified as follows *Pseudomonas aeruginosa* (2), *Escherichia coli* (65), *Staphylococcus aureus* (20), *Klebsiella pneumonia* and *Proteus mirabilis* (2) for each, *Scaphirhynchus albus, Morganella morganii* and *Micrococcus* (1), *Staphylococcus capitis, Staphylococcus epidermidis, Proteus vulgaris, Klebsiella oxytoca, Citrobacter freundii and Pseudomonas luteola* (1). Different degrees of sensitivity were shown by the isolates for different types of antibiotics. Antibacterial activity of aqueous *Z. officinale* extract was (7–30) mm while the alcoholic extract of *Z. officinale* was (4–20) mm. aqueous *A. sativum* extract (3–26) mm while alcoholic extraction (5–22) mm.

Keywords: *Zingiber officinale, Allium sativum*, Urinary tract infection

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INTRODUCTION

Urinary tract infection (UTI) is a common disease that causes referral of children to hospitals.[1] Urinary tract is a body filter system to remove liquids, or urine; consisting of kidneys, ureters, bladder and urethra.[2] UTI caused by many bacterial spices that could easily penetrate urinary system. Females get UTI more than males due to their urinary tract's design.[3] Variety of Enterobateraceae like *Escherichia coli*, *Proteus mirabilis, Pseudomonas aeruginosa*, *Acinetobacter bumanii*, *Serrati*, and *Morganella morganii* considers the common cause for UTI. Although gram positive bacteria, include *Enterococcus*, *Staphylococcus*, and *Streptococcus agalacticae* may also causes UTI.[4] Antibiotics, which are chemotherapy agents, have been used to treat bacterial infections associated with UTIs for years. But these pathogens are becoming more resistant to the available antimicrobials. Thus, it has become necessary to use substances with lethal activity or effective inhibitor of pathogens, *i.e.,* the use of new alternative medicine to reduce the spread of pathogens is an important part of drug development studies. Also, at the present time it is common to use herbal medicines in the treatment of many diseases caused by different types of bacteria, as these types are less resistant to natural products.[5]

MATERIALS AND METHODS

Collection of Samples

A 200 urine samples were collected from children under the age of 15 years old suffering from UTI during the period (6-1-2021 to 5-4-2022) from different hospitals. All samples were preserved in sterile containers.

Cultivation and Identification

Bacterial isolates were cultured on selective and differential media to see and identify their colonial morphology differences, and then gram staining was applied, followed by biochemical tests, which included (catalase, oxidase, urease, indole test, methylred, voges-proskauer, citrate utilization, TSI. The final diagnosis used API20E and Vitek-2 compact system.[6]

Antibiotic Sensitivity

By using Kirby Bauer or what is called the disk diffusion method, antibiotics susceptibility was carried to all isolates. The zone of inhibition was measured.[7]

**Author for Correspondence: ahmedalkubeisi79@yahoo.com*

Plants Collection

Both plants (Ginger and Garlic) were collected from the market. Both tap water and then distilled water were used to wash the plants, dried and smashed to powder and kept in the refrigerator for later use.[8]

Preparation of Extract

A 100 gms of the kept powder was dissolved with 1000 mL of solvents (ethanol alcohol and distilled water) for 72 hours to prepare alcoholic and aqueous extract.[9]

Disc Diffusion Method

To evaluate the bactericidal activity, Mueller Hinton agar was used. A sterile piece of filter paper were soaked with 500 µL of the extracts and then laid over the agar, which was inoculated with bacteria, the plates were then cultivated in the incubator. Distilled water rely as control and impinem used as standard to confirm that all the bacteria isolated tested were inhibited by the antibiotic. The antibacterial activity was measured by calculation inhibition zone.[10]

RESULTS

A 100 bacterial isolates were collected from a clinical sample of UTI patients for children, 60 (60%) female and 40 (40%) male (Figure 1).

The sample was collected from patients under 15 years old as shown in Figure 2.

The results have shown that *E. coli* (65) was found to be the dominant isolates from urine sample, *S. aureus* (20), *P. aeruginosa* (2), *K. pneumonia, P. mirabilis* (2) for each one, *S. albus, M. morganii and Micrococcus* (1) each of them. *S. capitis, S. epidermidis, P. vulgaris, K. oxytoca, C. freundii and P. luteola* (1) Figure 3.

All isolate were multidrug resistant against antibiotic. *Staphylococcus* isolates were resistance to amoxicillin/clavulanic acid, ampicillin, aztreomycin, meropenem, azithromycin, ceftazidime, ceftridacin, cyclodextrin, AT, ciprofloxacin, co-trimoxazole, cefixime, cephathiane and sensitive to amikacin, impinem, gentamicin, nalidixic acid , nitrofuration as shown in Figure 4. *E. coli* isolated were resistant to all antibiotics, but sensitive to nitrofuration, meropenem, aztreomycin, gentamicin, and cotrimoxazole. *Micrococcus* resistant to all antibiotics while it was sensitive to amoxicillin, clavulanic acid, ampicillin, aztreomycin, cephathiane, cefixime, ciprofloxacin, impinem, nalidixic acid and itrofuration. *M. morganii* resistance to all antibiotic while it was sensitive to ampicillin, aztreomycin, ciprofloxacin, azithromycin, gentamicin, impinim, meropenem and nitrofuration. *C. frenudii* isolates were resistant to all antibiotics and sensitive to aztreomycin, azithromycin, ceftazidime, ciprofloxacin, impinem, meropenem nalidixic acid, nitrofuration as in Figure 5. *K. pneumonia and K. oxytoca* Isolates were resistant to all antibiotic except aztreomycin, gentamicin, meropenem, nalidixic acid, nitrofuration and Impinem they were sensitive for them as shown in (Figure 6). *P. aeruginosa* and *P. luteola* showed sensitive only to amikacin, ciprofloxacin, gentamicin, and meropenem and impinem as shown in (Figure 7). *P. mirabilis* and *P. vulgaris* show a resistance to all antibiotic except aztreomycin, ciprofloxacin, meropenem they were sensitive to them (Figure 8).

One bacterial isolate was selected from one of the species that was isolated from cases of UTI from children in order to investigate the susceptibility of the aqueous extract of *Z. officinale* with different concentrations, which were (0.125, 0.25, 0.5, 0.75, 1%), the results of *Z. officinale* aqueous extract effectiveness showed at (0.125%) concentration, it is ineffective in inhibiting *E.coli, S. capitis, S. epidermidis, P. mirabilis, P. vulgaris, K. pneumonia, K. oxytoca*, *Micrococcus, P. aeruginosa and P. luteola*, the diameter of the inhibition zone was (0) mm, while the diameter of the inhibition zone for *S. albus, S. aureus*, *M. morganii, C. freundii* was 5, 3, 4, 3 mm, respectively. The (0.25%) concentration was not effective in inhibiting *E. coli, S. epidermidis, P. mirabilis, K. pneumonia, K. oxytoca, Micrococcus, P. aeruginosa* and *P. luteola;* the diameter of the inhibition zone was 0 mm, the minimum inhibition diameter for *P. vulgaris* at the same concentration was 3 mm, and the highest inhibition diameter was 9 mm for *S. albus*. The concentration of 0.5% was effective in inhibiting all bacterial isolates except for four bacterial isolates (*S. epidermidis, K. oxytoca, Micrococcus*, and *P. luteola*), the diameter of the mentioned isolates was 0 mm, while the diameter of the inhibition for the rest of the isolates ranged from 3–18 mm. The concentration of 0.75% showed efficiency in inhibiting all bacterial isolates except for *Micrococcus*, the minimum diameter of inhibition was 3 mm, while the highest diameter of inhibition was 24 mm at the same concentration. The concentration of 1% was the best for the aqueous extract of *Z. officinale*, the lowest zone of inhibition was 5 mm for *P. luteola*, while the highest zone of inhibition was 30 mm for each of *S. aureus* and *M. morganii*, while *Micrococcus* were also resistant to the same concentration with 0 mm inhibition zone as shown in Figure 9.

The results of testing the activity of *Z. officinale* alcoholic extract at a concentration of 0.125% showed that it is not effective in inhibiting all selected bacterial isolates, except for *S. albus* and *S. aureus*, the diameter of them was 4 mm after treating them with alcoholic extract, while the inhibition zone of *M. morganii* was 5 mm. The 0.25% concentration was not effective in inhibiting *E. coli, S. epidermidis, P. mirabilis, P. vulgaris, K. oxytoca, Micrococcus,* and *P. luteola*, the diameter of the inhibition zone was (0) mm for the previous isolates, while it was minimum diameter of inhibition of *K. pneumonia* at the same concentration was 4 mm, and the maximum diameter of inhibition was 10 mm in *M. morganii*. The concentration of 0.5% was effective in inhibiting all bacterial isolates except *Micrococcus* and its diameter of inhibition was 0 mm, while the diameter of the inhibition zone of the rest of the isolates ranged from 4 to 16 mm. The concentration of 0.75% showed efficiency in inhibiting all bacterial isolates except for *Micrococcus*, as the lowest diameter of inhibition was 4 mm, while the highest diameter of inhibition at the same

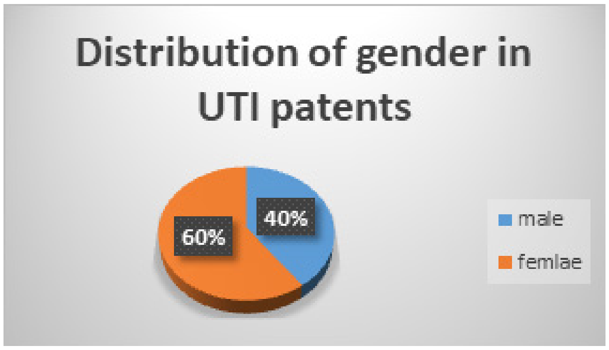

Figure 1: Distribution of UTI patients under.

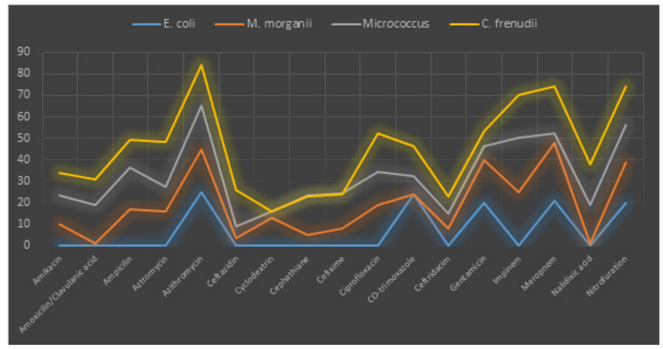

Figure 5: Antibiotic activity against *E.coli, M. morganii, Micrococcus* and *C. frenudii.*

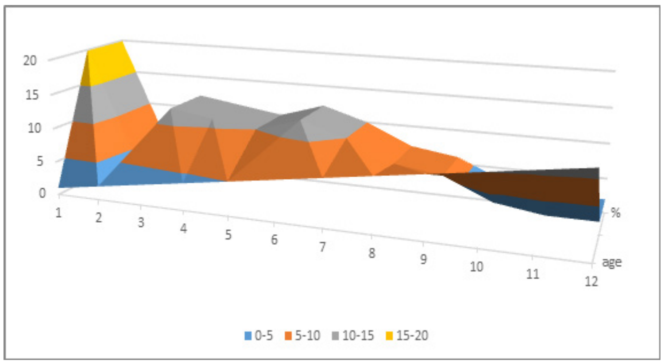

Figure 2: Distribution of Age in patients.

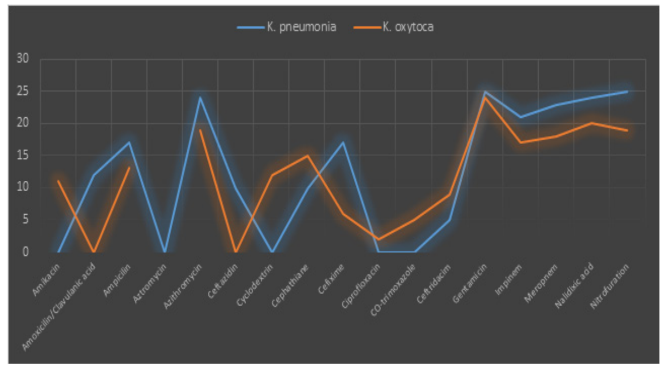

Figure 6: Antibiotic activity against *K. pneumonaie* and *K. oxytoca*

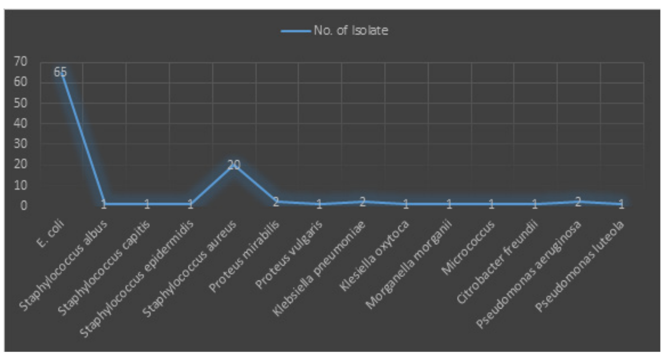

Figure 3: Number of bacterial isolate in urinary tract infection patients.

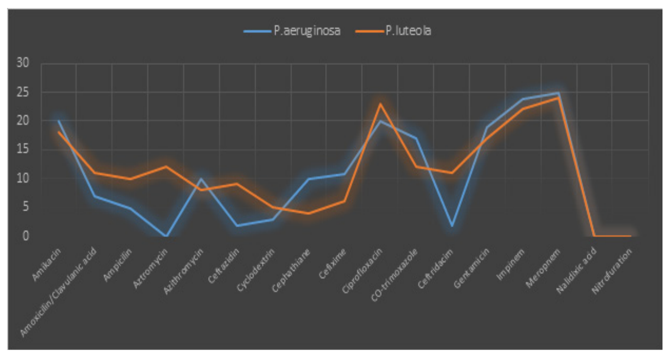

Figure 7: Antibiotic activity against *P. aeruginosa* and *P. luteola.*

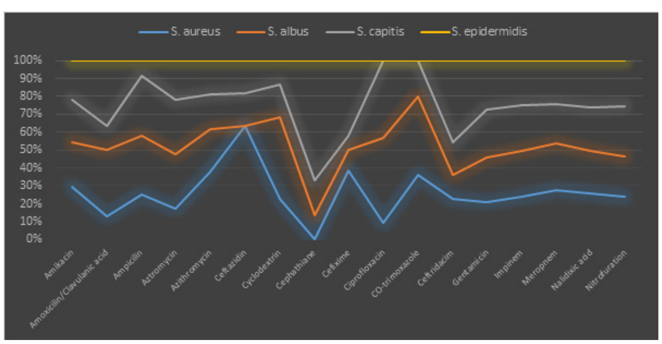

Figure 4: Antibiotic activity against *Staphylococcus sp.*

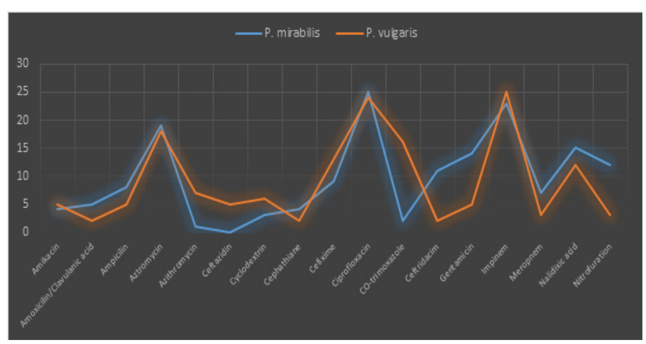

Figure 8: Antibiotic activity against *P. mirabilis* and *P. vulgaris.*

concentration was 18 mm. The lowest inhibitory diameter was 5 mm for *Micrococcus*, while the highest was 20 mm for *S. aureus, P. vulgaris* and *P. aeruginosa* at a concentration of 1%. It was clear from the previous results that the aqueous extract of ginger is more efficient than the alcoholic extract in inhibiting bacteria, and the effectiveness of the two extracts varies according to the concentration and type of bacteria as shown in Figure 10.

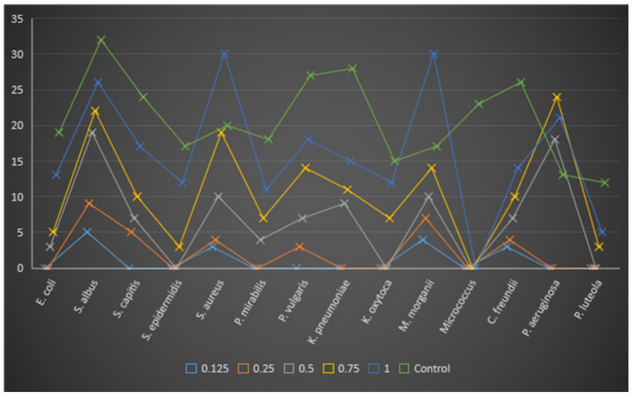

Figure 9: Antimicrobial activity of aqueous extract of *Z. officinale*.

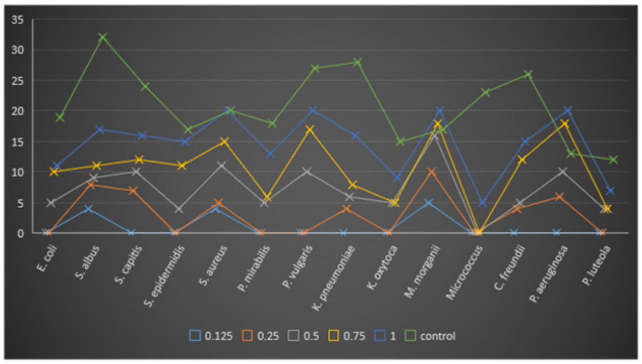

Figure 10: Antimicrobial activity of alcoholic extract of *Z. officinale.*

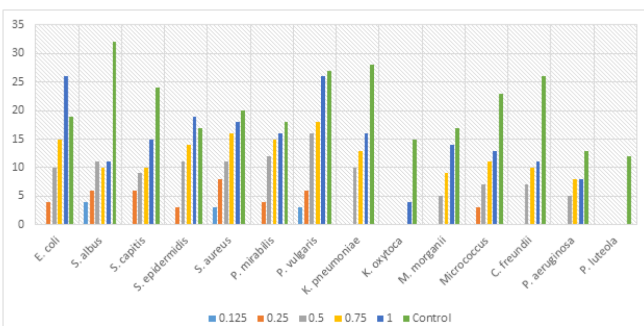

Figure 11: Antimicrobial activity of aqueous extract of *A. sativum*.

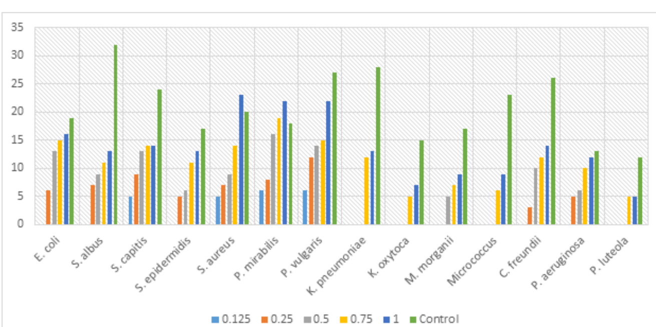

Figure 12: Antimicrobial activity of alcoholic extract of *A. sativum.*

The previous isolates were selected again in order to know the effect of *A. sativum* on bacteria isolated from cases of urinary tract infection in children. They were treated with the same previous concentrations of the aqueous and alcoholic extract of *Z. officinale*. The results of testing the effectiveness of the *A. sativum* water extract at a concentration of 0.125% showed that it is not effective in inhibiting all bacterial isolates, except for *S. albus, S. aureus* and *P. vulgaris*, the diameter of the inhibition zone was 4, 3, 3 mm, respectively. The concentration 0.25% was not effective in inhibiting *K. pneumonia, K. oxytoca, M. morganii, C. freundii, P. aeruginosa* and *P. luteola*, and the diameter of the inhibition zone was 0 mm, while the lowest diameter of inhibition for *S. epidermidis* at the same concentration was 3 mm and the highest inhibition diameter was 8 mm for *S. aureus*. The concentration of 0.5% was effective in inhibiting all bacterial isolates except for two bacterial isolates *K. oxytoca* and *P. luteola*, the inhibition zone diameter of the mentioned isolates was 0 mm, while the diameter of the inhibition for the rest of the isolates ranged from 5 to 16 mm. The concentration of 0.75% showed efficiency in inhibiting all bacterial isolates except for *K. oxytoca* and *P. luteola*, as the lowest inhibition diameter was (8) mm, while the highest inhibition diameter reached (18) mm at the same concentration. The concentration of 1% was the best, it gives (4) mm which was the lowest inhibitory diameter for *K. oxytoca*, while the highest inhibitory diameter was (26) mm for each of *E. coli* and *P. vulgaris*, although *P. luteola* show resistant to the same Figure 11.

Alcoholic extract of *A. sativum* at a concentration of 0.125% showed that it was not effective in inhibiting all selected bacterial isolates, except for *S. capitis, S. aureus*, *P. mirabilis* and *P. vulgaris*, the inhibition zone diameter of *S. capitis* and *S. aureus* was 5 mm, while the inhibition zone of *P. mirabilis* and *P. vulgaris* was 5 mm. The 0.25% concentration was not effective in inhibiting *K. pneumoniae, K. oxytoca, M. morganii, Micrococcus,* and *P. luteola*, the diameter of inhibition zone was 0 mm for the previous isolates, while it was minimum diameter of inhibition of *C. freundii* at the same concentration was 3 mm, and the maximum diameter of inhibition was 12 mm in *P. vulgaris.* The concentration of 0.5% was effective in inhibiting all bacterial isolates except *K. pneumoniae, K. oxytoca, Micrococcus,* and *P. luteola* and its diameter of inhibition was 0 mm, while the diameter of the inhibition zone of the rest of the isolates ranged from 5 to 14 mm. The concentration of 0.75% showed efficiency in inhibiting all bacterial isolates, the lowest diameter of inhibition was 5 mm, while the highest diameter of inhibition at the same concentration was 19 mm. The lowest inhibitory diameter was 5 mm for *P. luteola*, while the highest inhibitory diameter was 23 mm for *S. aureus* concentration of 1%. It was clear from the previous results that the Alcoholic extract of *A. sativum* is more efficient than the aqueous extract in inhibiting bacteria, and the effectiveness of the two extracts varies according to the concentration and type of bacteria, as shown in Figure 12.

DISCUSSION

UTI occurred in females more than males in another study showed females 63.6 and 36.4% male, It has been observed that the rate of the disease is much higher in females than in males, also the results from[11] agrees with previous information. Antibiotic provides therapy for bacterial infection, back to the discovery of antibiotics, they were and still used as chemotherapeutic material that could lead to the eventual eradication of infectious disease. But worldwide the resistant bacteria has become a major therapeutic problem in future, In addition multidrug resistant strains, they may be isolated from community-acquired infections.[12] *Staphylococcus sp.* were resistant to amoxicillin/clavulanic acid and ampicillin. In contrast, the were sensitive to amikacin, impinem, gentamicin, nalidixic acid, nitrofuration, also *Proteus* sp. showed a resistance to aztreomycin, ciprofloxacin, meropenem. In contrast, *Pseudomonas sp.* showed sensitivity only to amikacin, ciprofloxacin, gentamicin meropenem and impinem. *Klebsiella* sp. were resistance against all antibiotic while sensitive against aztreomycin,gentamicin, meropenem, nalidixic acid, nitrofuration and impinem. These result agreed with,[13] in which they refer to that, the bacteria which were isolated from UTI, were multi drug resistant to ampicilin, amoxicilin, ceftizoxime, cefepime, tetracycline, and this resistance may came from efflux pumps.[14] All isolates have the ability to resist variety types of antibiotics (multidrug resistant). *E. coli*, *Micrococcus* and *M. morganii* were resistant many types of antibiotics, also they were sensitive to meropenem, nitrofuration, gentamicin, aztreomycin, amoxicillin, clavulanic acid, impinem and co-trimoxazole. Antimicrobial activity of aqueous extract of *Z. officinale* gives an inhibition zone between 7–30 mm, while there was no effect on *Micrococcus*, also, alcoholic extract gives an inhibition zones between 4–20 mm. Aqueous extract of *A. sativum* gives an inhibition zone to 3–26 mm. No effects of these extract against *P. aeruginosa*, *K. oxytoca* and *Micrococcus*. The inhibition zones of alcoholic extract ranged between 5–22. Results from,[15] reviled that *Z. officinale* and *A. sativum* has an activity to inhibit *E. coli* growth and formation of biofilm, also a recent study by,[16,17] point to the activity of *Z. officinale* to inhibit growth of *S. aureus*, *P. aeruginosa* and *P. vulgaris.* The activity of *Z. officinale* due to the presence of zingiberene, which is the main component in the plant and is the active compound with the aid of sesquiterpenoids, and trace monoterpenoid fraction, (β-sesquiphellandrene, cineol and citral).[18,19] The study of[20] reports that the water and alcoholic extract of *Z. officinale* were both effective against *S. aureus.* Also, our results agreed with,[21] indicating that *Z. officinale* is a very useful material against multidrug resistant bacteria. *A. sativum* is very useful against mult-drug resistance bacteria like *E. coli, K. pneumonia, E. faecalis, and P. mirabilis* that causes UTI infection, the activity of *A. sativum* extract differ from bacteria to another depending on the concertation.[22,23]

CONCLUSION

The activity of aqueous and alcoholic extract of *Z. officinale* and *A. sativum* depends on the concentration of the extract and the type of isolate. Aqueous extract of *Z. officinale* was more effective than alcohol. On the contrary, the alcoholic extract of *A. sativum* was more effective than aqueous extract. By comparing the result of both extract with the activity of antibiotics, it seems that is some cases these extract may be useful in treating the case, but in other cases the didn't have any activity against bacteria, and this may come from the resistant of the bacteria itself to the active compounds of the plants, and this occur as a result from taking antibiotics incorrectly casing mutations in the bacterial genome and make them very Immune for any medications.

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