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Antimicrobial activity of two plant extracts against bacterial pathogens isolated from patient with urinary tract infection

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KEY WORDS:
Urinary tract infections, plant extracts , pathogenic bacteria.

ABSTRACT
This study included isolation and diagnosing some bacterial species that cause urinary tract infections and testing the effect of aqueous and alcoholic extracts of garlic and ginger fruits on it . A number of urine samples (200) were collected and taken from patients and patients at Azadi Hospital, for the period 18/11/2021 to 10/3/2022, and included males and females for ages between (15-60) years, with a ratio of (70%fmales and (30%) males After planting the samples on blood agar, MacConkey agar, and Mannitol salt agar media, (150) samples of them gave bacterial growth when cultured, with a rate of (75%), as it gave two types of growth, negative for gram stain (73.3%), and positivefo Gram stain (26.7%), while the other 50 samples did not give any bacterial growth. The infection rate was higher among females (70%), while males (30%), and the highest infection rate was recorded in the age group (15-30) years in both sexes. Bacterial isolates were isolated and diagnosed based on their morphological characteristics an111d biochemical tests. The following species appeared: *Escherichia coli* 45 isolates (30%), *Klebsiella pneumonia* 25 isolates (16.7%), Staphylococcus aureus 40 isolates (26.6%), Pseudomonas aeruginosa 15 isolate (10%), Proteus mirabilis 25 isolate (16.7%). The effectiveness of aqueous and alcoholic extracts was tested at concentrations (25%), (50%) and (100%), where the alcoholic extract was more efficient compared to the aqueous extract and the concentration was (100%) is the most effective concentration on bacterial isolates and the least at the extract at a concentration of (25%) for the aqueous or alcoholic extract..

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the human intestine. It is an anaerobic Gram-negative bacterium and belongs to Enterobacteriaceae. Other Gram-negative bacterial causes include Proteus spp , Klebsiella spp and Pseusdomonas . There is Gram-positive Staphylococcus spp. Normal urine contains salts, ions, and acids, including uric acid , and does not contain bacteria. Urinary tract infection occurs when bacteria enter the bladder and multiply there (2).The wrong and repeated use of chemical treatments against pathogens has led to the creation of many alternatives, including medicinal plants and herbs, due to their effectiveness against microorganisms. The use of medicinal herbs has increased recently due to the side effects and the exorbitant cost of chemical drugs.

The emergence of microbial strains resistant to antibiotics prompted many researchers. To find alternative anti-bacterial materials and separate their effective components, as medicinal plants contain special chemicals, and the most important of these materials are alkaloids, volatile or aromatic oils, gum, light fats, and phenols.

MATERIALS AND METHODS

Culture media that were used during the current study are shown in Table 1

Table 1: Culture media and its manufacturer

No	The middle	The manufacture company
1	Blood agar base	Mast (England)
2	MacConkey agar	Oxiod (England)
3	Mannitol salt agar	Oxiod (England)
4	Nutrient agar	Mast (England)
5	Muller-Hinton	Oxiod (England)
6	Peptone water	Oxoid (England)
7	Simmon Citrate agar	Mast (England)
8	Brain _heart infusion agar	Oxoid (England)
9	Kligler Iron agar	Himedia(India)
10	Eosine methylene blue	Oxoid (England)
11	MR-VP	Difco(USA)
12	Nutrient broth	Mast (England)
13	*Ps. aeruginosa* medium	Himedia laboratory (india)
14	Urea agar base	Oxiod (England)

Sterilization Methods

Moist-heat sterilization method was used to sterilize the culture media and the solutions used in the study by placing them in the autoclave at a temperature of 121 ◦C and a pressure of 1.5 pounds ing2 for a period of 15 minutes. In the electric oven, at a temperature of 180-200 ◦ C for a period of two hours, sterilization methods were used by filtration using filter papers with a diameter of (0,22- 0.45) micrometers to sterilize solutions such as urea solutions (Cheesbrough 2006).

INTRODUCTION

Urinary tract infections (UTIS) is an inflammatory response that occurs in the urinary system as a result of the colonization of microbial pathogens of the sterile urinary tract. Bacterial infections occupy the first place among other pathogens, as 95% of urinary tract infections are caused by bacteria, compared to only 5% caused by other causes. Urinary tract infections are in second place after respiratory tract infections. The infection occurs along the urinary tract, as urinary tract infections constitute 23% of the total bacterial infections in Iraq for different age groups and for both sexes, but females are more susceptible to infection (1) and the reason for its prevalence among women is the proximity of the anal opening to the opening of the anus.

The urethra, which facilitates the entry of bacteria into the urinary tract, and this is very common in women due to the anatomical structure of the female urinary system, as the shortness of the urethra and its close proximity to the source of infection is the reason for the high percentage of infections in females, especially since the most bacterial species that cause urinary tract infection are E. coli, which is responsible for 90% of the causes of urinary tract infection and its source is

Preparation of Culture Media

The culture media was prepared according to the manufacturer's instructions installed on the bottles, the media was sterilized, then left to cool, then the media was poured into the dishes inside the hood, then incubated at a temperature of 37 °C for 24 hours before use to ensure that it was not contaminated, then kept in the refrigerator at 4 °C until use (3) The media includes:

Blood Agar

This medium was prepared according to the manufacturer's instructions, as shown in a study conducted by (4)

MacConkey Agar

This medium was prepared according to the manufacturer's instructions, as shown in a study conducted by (5)

Nutrient Agar

This medium was prepared according to the manufacturer's instructions, as shown in the study carried out by him (6)

Nutrient broth

This medium was prepared according to the manufacturer's instructions, as shown in the study carried out by him (7)

Eosin- methylene blue agar

This medium was prepared according to the manufacturer's instructions, as shown in the study carried out by him. (8)

Mannitol Salt agar

This medium was prepared according to the manufacturer's instructions, as shown in the study carried out by him. (9**)**

Muller-Hinton

This medium was prepared according to the manufacturer's instructions, as shown in the study carried out by him. (10)

Simmon's Citrate agar

This medium was used in the (IMVIC) tests: Indole-Methyl red- Voges proskauer Citrate to differentiate between the types of bacteria of the intestinal family (11)

Peptone Water

This medium was used to differentiate between bacteria of the intestinal family that have the ability to ferment glucose or lactose and liberate sulphides with or without gas (12)

Kligler Iron agar

This medium was prepared according to the manufacturer's instructions and used in the indole test (one of the IMVIC series tests) (13)

MR_ VP media

This medium was prepared according to the manufacturer's instructions and was used to investigate the ability of bacterial isolates to fully or partially hydrolyze sugars and produce acid (14)

Brain-heart infusion agar

This medium was prepared according to the manufacturer's instructions, and was used to activate the isolated bacteria after a period of isolation (15).

***Collection of samples**

The work was conducted in the laboratories of Azadi Hospital in Kirkuk and the laboratories of the Department of Life Sciences, College of Education for Pure Sciences / University of Kirkuk. A number of urine samples (200) were collected and taken randomly from sick persons in Azadi Hospital. Samples were taken of varying ages and from both sexes. From males and females, and for the period between 11/18/2021 to 3/10/2022, urine samples were taken and placed in sterile plastic containers, part of which was placed in a centrifuge, microscopically examined, and cultured.

***Cultivation of Specimen**

After collecting the samples, they were planted on the culture media by taking a drop of the urine and spreading it on the surface of the medium and planting it on different media, including blood and Maconky medium, and incubated at 37°C for 24 hours. 4 'm until the diagnostic tests are performed, and the diagnosis was made according to the methods used that he conducted

***The isolates under study were identified according to the following tests:**

1- Morphological Diagnosis 2-Microscopical Diagnosis (3-Biochemical tests

***Preparation of Water Extracts**

Weighed 75 g of the plant sample and then placed it in a glass beaker containing 750 ml of sterile distilled water. The beaker was left on the shaker for 24 hours, after that the mixture was filtered using several layers of medical gauze to remove the large plant parts stuck in it, filtered again using leaves Millipor filtration to prevent the passage of impurities with the filtrate. Then the mixture was placed in an electric oven at a temperature of 40 °C until all the water evaporated and a dry powder was deposited at the bottom. The dry extract was kept in clean, well-sealed bottles in the refrigerator at a temperature 3esx

The alcoholic extract was prepared using the same method as the aqueous extract with the same weights, except for replacing the distilled water with ethyl alcohol at a concentration of 76%.

***Testing the inhibitory effectiveness of plant extracts against bacteria**

I followed the method (17) to find out the ability of bacteria to resist these extracts. This method is summarized as follows:

1- Mueller-Hinton medium was prepared, poured into dishes and left to solidify, then inoculated by placing 0.1 milliliter of bacterial suspension by means of a pipette in the middle of the dish and spreading by means of a glass diffuser to cover the surface of the agar.

2- Small holes were made in the dishes through the corkscrew, with a diameter of 5 mm, and a regular distance between them, and the number of holes in one dish was three.

3- microliters of extract were added to each hole at successive concentrations of 100, 75 and 25, and the medium was left to dry and the liquid spread inside the hole, then it was placed in the incubator for 24 hours at a temperature of 37 °C.

4- The results were read by measuring the diameter of the transparent halos that form around the hole using a standard ruler. As for the appearance of bacterial growth and the absence of a halo, this indicates that the results are negative and the extract has no effect on the bacteria.

RESULTS

samples of them gave bacterial growth when cultivated150 on the culture media, with a rate of 75% of the total number of urine samples, while the remaining 50 samples, at a rate of 25%, did not give any bacterial growth. Gram-negative bacteria at a rate of 73.3%, and this result is similar to what was reached by (18) in Tikrit, which recorded 65.6% of gram-negative bacteria and 34.4% of gram-positive bacteria. Thus, the gram-negative bacteria for those infected had the highest percentage of the gram-positive bacteria.

Diagnosis: Bacterial isolates isolated from urine were diagnosed by several approved methods, including: Phenotypic and microscopic diagnosis of bacterial isolates causing urinary tract infections The results of the phenotypic and microscopic tests (using a gram stain) that were conducted on the growing bacteria showed that all the gram-negative bacteria are rod-shaped and grow on McConkey's medium, which does not allow the gram-positive bacteria to grow on it. E.coli colonies appeared pink on MacConkey's medium as a result of fermentation of lactose sugar (Figure 1A), and E.coli colonies when cultured on EMB medium showed a brig---ht metallic green color (Figure 1B). These bacteria are (19).

As for K. pneumonia, its colonies appeared in pink color on McConkey Acar medium (Figure 2A), while its colonies appeared in dark violet color on EMB medium as in (Figure 2B). Colonies of P.aeruginosa bacteria also appeared and were characterized by a bluish-green color on the medium of the nutrient agar (Figure 3A), while they appeared on the medium of the MacConkey agar of a pale color (Figure 3B)(.)20)

Figure 1B

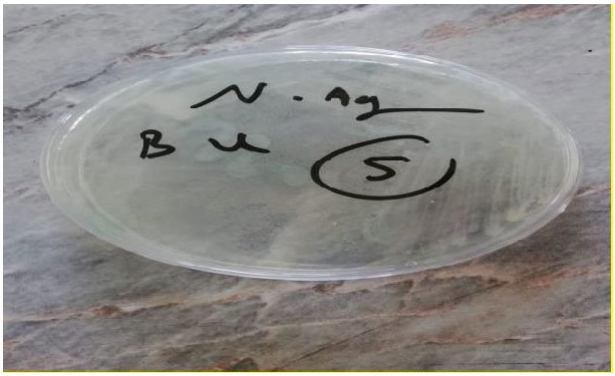
Figure 1A

E.coli colonies incubated at 37°C
A - on MacConkey agar medium
B - on EMB agar medium

Figure 2B

Figure 2A

A- On McConkey medium Klebsiella pneumonia novae incubated at 37°C B- On EBM medium



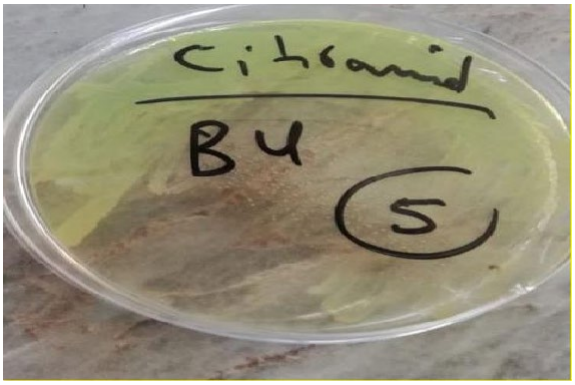


Figure 3B Figure 3A

A- On the agar medium feeding P. aeruginosa colonies incubated at a temperature of 37 C.

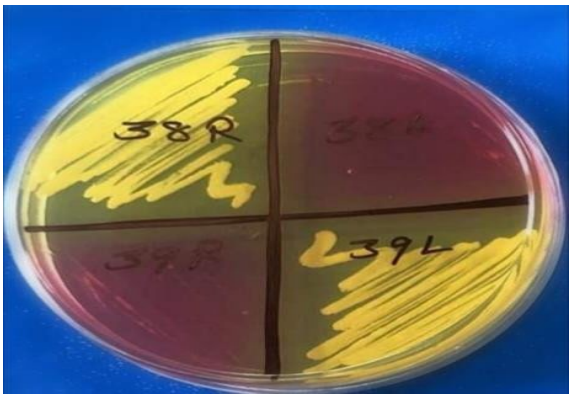
B- On the medium of Makonk

.P. mirabilis bacteria were detected on Nutrient agar medium as in (Fig. 4), and they are pale

Figure 4) colonies of P. mirabilis on the nutrient agar medium kept at a temperature of 37%

As for the gram-positive S.auerus bacteria, it was distinguished by its spherical shape. It gave a white color when it grew on blood vessels, and the color of the medium changed from pink to golden yellow, indicating that the growing bacteria (Fig. 5). The pH function of neutral to acidic media and its sensitivity to anti-novobiocin (Fig. 6) and blood analyzer on agar medium

(Figure 6) Sensitivity of S. aureus to novobiocin on Acar medium



(Figure 5) S.auerus on a medium Mannitol salt agar

Biochemical diagnosis of isolates

The results of the biochemical tests were recorded to identify the species and species of Gram-positive and Gram-negative bacteria that were isolated, which included K. pneumonia, P. aeruginosa, E.coli, S. aureus, P. mirabilis. These tests included oxidase, catalase, urease, growth on iron-triglyceride medium and IMViC tests.

(Table 2) shows the biochemical tests for Gram-negative isolates, as E. coli gave a positive result as shown in the table. As for K. pneumonia, it gave a negative result for my test. Ps bacteria. aeruginosa positive. P. mirabilis was characterized as negative for Gram staining.

Table 2: Biochemical tests for Gram-negative isolates

Triple-Sugar Iron				Urease	Citrate	Voges proskouer	Methyl – red	Indol	Catalase	Oxidase	test type / isolated bacteria
Gas	H_2S	Butt	Slope								
+	-	A	A	-	-	-	+	+	+	-	**E.Coli**
+	-	A	A	+	+	+	-	+	+	-	**K pneumonia**
-	-	K	K	-	+	_	-	-	+	+	**P aeruginosa**
+	+	A	K	+	+	V	+	_	+	_	**Pmirabilis**

(-) Negative test (+) positive test A=Acid K=Alkalain

(Table 3) shows the biochemical tests for Gram-positive isolates. S. aureus

Water+ alcohol	Alcoholic	Watery	Abstracts

Table 3: Biochemical tests for Gram-positive isolates

Novobiocin	Mannitol Salt Agar	Haemolysis	Oxidase test	Coagulase test	Catalase test	test type / bacteria staphylococcus
S	+	+	_	+	+	***S. Aureus***

S=Senstive (+) positive test (-) Negative test

***Bacterial isolates causing the disease and their isolation ratios**

After culturing the samples on Blood agar medium, MacConkey agar medium, and Mannitol Salt agar medium, then they were isolated and diagnosed by the mentioned methods.

Table 4: Numbers and percentages of bacterial species isolated from urine

(%)	number of isolates	isolated bacteria
30%	45	Escherichia coli
26.6%	40	Staphylococcus aureus
16.7%	25	Klebsiella pneumonia
10%	15	Pseudomonas aeruginosa
16.7%	25	Proteus mirabilis
100%	150	the total

It is clear from the above table4 that the most isolated bacterial species in this study is E. coli, with an isolation rate of 30%, while this percentage differed with what was stated by (21), which was 11% who conducted a study on more than 2000 patients suffering from urinary tract infections In several hospitals in Ireland, the percentage of E. coli isolation was only 14.4%. It shows the effect of the environment and the study area on the research results. The high percentage of infection with this bacteria is caused by the bacteria leaving the intestines, causing contamination of the area around the vagina, including the urinary tract, causing inflammation of the tract, which is helped by its possession of cilia. The bacteria that stick to the lining of the urinary tract, S. aureus ranked second among the pathogens of urinary tract infections, with a rate of 26.6%. K. Pneumoniae came in the third place, with an isolation rate of 16.7%, because this bacterium possesses a portfolio located on the surface of the bacterial cell that protects it. From the body's immune response and dehydration. P. aeruginosa was also isolated, where it was isolated by 10%, as the bacteria possess biofilms, adhesion mechanisms, antibiotic resistance, and low nutritional requirements.

***The effect of garlic extract on the growth of bacterial isolates:**

The current study of the aqueous and alcoholic extract showed a "clear" difference in the inhibition of the bacterial species under study, as the alcoholic extract of Allium sativum was more inhibition than the aqueous extract by observing the diameter of the inhibition in the concentrations 25%, 50%, 100%, as shown in Table (5).

Table (5) Effect of aqueous, alcoholic and synergistic extract of garlic plant on the growth of bacterial isolates (mm).

Concentrations (100%									
100	middle isolation	**100 %**	**50 %**	**25 %**	middle isolation	**100 %**	**50 %**	**25 %**	
	14.0 A	**14**	**14**	**10**	**11.3 A**	**14**	**12**	**8**	***E. coli***
	15.7 A	**20**	**15**	**12**	**8.7 B**	**12**	**14**	**0**	***S. Aureus***
	12.7 B	**61**	**12**	**10**	**7.3 B**	**12**	**10**	**0**	***P. mirabilis***
	15.3 A	**22**	**13**	**11**	**10.7 A**	**14**	**10**	**8**	***K. pneumonia***
	11.3 B	**22**	**1**	**11**	**8.0 B**	**14**	**10**	**0**	***P. aeruginosa***
		19.6 A	**11.0 B**	**10.8 B**		**13.2 a**	**11.2 B**	**3.2 C**	**average concentration**

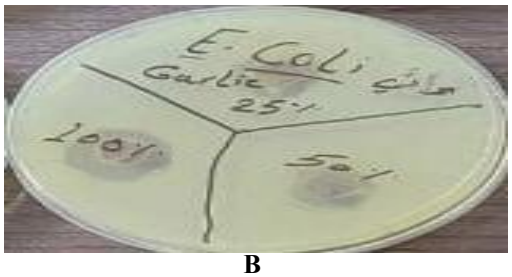
The results of the alcoholic extracts of the garlic plant also showed a difference in the extent of the inhibition of the bacteria under study. The broad antibacterial effectiveness of garlic is due to its containment of sulfur compounds, including Allicin, Thiosulfinates. Allicin is transformed into organic sulfur compounds, the most important of which are Diallyltrisulfide, di-allyldisulfide. Allicin acts on partial inhibition of DNA and protein production and total inhibition of RNA production.The presence of allicin has an effect on the oxidation of enzymes.These compounds have anti-microbial activity against the growth of non-sulfur compounds such as Vitamin B, proteins, iron and saponins.

The results agree with (22), which concluded that the effect of the alcoholic extract is higher than that of the aqueous extract. The reason for the effectiveness of the garlic extract is attributed to its containment of alkaloids, saponins, and terpenes. The action of the alkaloids is to stop the manufacture of nucleic acids in the microscopic living cell through the action of the co-enzyme produced by the bacterial cell, while the effect of the saponins is due to the removal of the membranes of the microorganisms as it works to analyze the living .)cells(23)

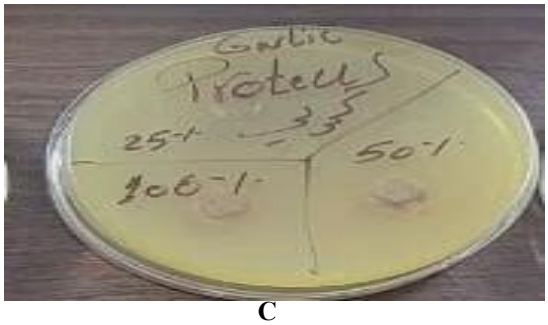
A

A

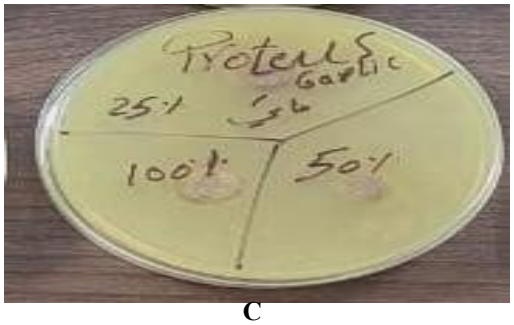
B



B



C



C

D D

E E

B- alcoholic A-watery

(Fig. 5) The sensitivity of bacterial isolates to garlic extract

for the following **A-S. aureus,B-E.coli, C-P.mirabilis, *D-Ps.aerioginosa , E-K. pneumonia types***

***Effect of ginger plant extract on bacterial isolates**

The current study of the aqueous and alcoholic extract, as in the case of the garlic plant extract, showed a difference in the inhibition of the bacterial species under study, as the alcoholic extract of Zingiber officinale Rosc Zingiber was more inhibition than the aqueous extract by observing the diameter of inhibition in concentrations 25%, 50%, 100%.

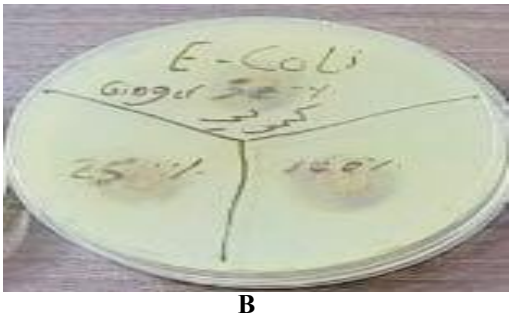
Table (6) The effect of the aqueous, alcoholic and synergistic extract of the Nesbis plant on the growth of bacterial isolates (mm)

Water+ alcohol		**Alcoholic**				**Watery**			**Abstracts**
Concentrations (100%									
100	middle isolation	**100 %**	**50 %**	**25 %**	middle isolation	**100 %**	**50 %**	**25 %**	**bacterial isolates Three isolates of each species**
	15.3 A	**18**	**16**	**12**	**12.0 A**	**14**	**12**	**10**	***E. coli***
	16.0 A	**20**	**16**	**12**	**13.3 A**	**16**	**14**	**10**	***S. Aureus***
	10.0 C	**16**	**10**	**4**	**6.0 C**	**10**	**8**	**0**	***Proteus mirabilis***
	13.3 B	**18**	**14**	**8**	**9.3 B**	**12**	**8**	**8**	***K. pneumonia***
	13.3 B	**18**	**14**	**8**	**7.3 C**	**12**	**10**	**0**	***P. aeruginosa***
		18.0 A	**14.0 B**	**8.8 C**		**12.8 A**	**10.4 B**	**5.6 C**	**average concentration**

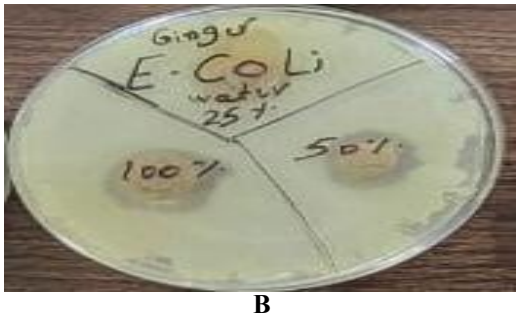
The results showed that the alcoholic extract of ginger is more effective against the growth of the bacterial species used in the current study than the aqueous extract of ginger, and this result is consistent with (24) in Tikrit, as the alcoholic extract of the roots of the ginger plant is distinguished by its containment of Zingiberene and farnescene as the two major components, in addition to containing Gingerone, bisabolene, geranionl, phellandrene, and Curcumene-β. As for the alcoholic extract of ginger, the same study indicated that it contains sterols, flavonoids, amino acids, carbohydrates, alkaloids, and glycosides, with small amounts of phenols and tannins, and the absence of all of the terpenes, lignins, and saponins.

A

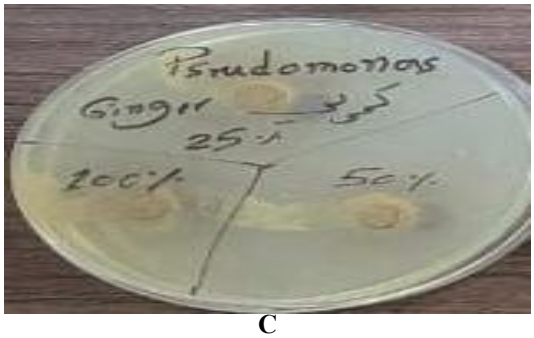
A



B



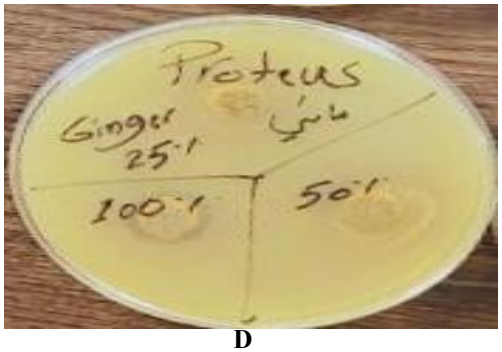
B



C

C

D



D

E

E

B- alcoholic y

A-water

Figure (6) Susceptibility of bacterial isolates to ginger extract

A-*S. aureus*,B-*E.coli, C-Ps.aerioginosa D- P.mirabilis , E-K. pneumonia*

Conclusions

1- The study showed that the most common types of Gram-negative and Gram-positive bacteria in urinary tract infections are E.coli, then S. aureus, followed by other bacterial species.
2- The incidence of urinary tract infections was higher in females than in males, and the highest incidence in both sexes was in the age group (15-30).
3- There are several factors that increase the chance of developing urinary tract infections, the most important of which are pregnancy, diabetes, area of residence, educational level, and sexual activity.
4- An increase in the level of bacterial resistance to antibiotics. Most of the bacterial isolates that carried the characteristic of resistance to the antibiotics used were affected by the plant extracts under study, with diameters of inhibition reaching 22 mm at a concentration of 100%, as in the bacteria Ps. aeruginosa and K. pneumoni, and less than that at concentrations of 50. % and 25%, Proteus mirabilis bacteria were the most resistant bacterial species to the extract, and alcoholic extracts showed higher effectiveness on bacterial isolates compared to aqueous extracts.
5- Garlic and ginger fruits contain biologically active substances such as flavonoids, amino acids, carbohydrates, and glycosides, with the absence of sterols, tannins, phenols, terpenes, alkaloids, lignin, and saponins, which have shown antibacterial activity against bacterial isolates, especially antibiotic-resistant bacteria.

Recommendations

1- Emphasis on conducting an antibiotic sensitivity test against bacteria isolated from patients with urinary tract infections, taking into account the selection of the most efficient and optimal antibiotic for treatment and avoiding randomness in the use of antibiotics.
2- Intensifying studies regarding the preparation of plant extracts of garlic, ginger, and other medicinal plants, and testing their inhibitory effectiveness against bacteria that cause urinary tract infections and those isolated from various sources.
3-Expanding the study of the inhibitory effect of ginger and garlic fruit extracts on other types of pathogenic microorganisms that cause urinary tract infections, such as parasites and fungi.
4- Separating the active substances present in garlic and ginger fruits and studying their effect on bacterial isolates individually or in a synergistic manner.
5- Conduct future studies to ensure the safety of using plant extracts and their active ingredients and without the appearance of side effects when used in treating urinary tract infections.
7- Paying attention to the development of medicinal and aromatic plants in general, and the plants of our current study in particular, and studying the wild medicinal plants that grow in our Arab world, collecting information about them and researchers taking an interest in them, and also guiding farmers on the methods of growing and caring for them.
8- Continuous health awareness of personal hygiene to get rid of bacterial infections that affect the urinary tract and other places in the body and the dangers of neglecting to treat them, as it leads to serious consequences.

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