



## Study Effect of the Aqueous and Alcoholic Extracts of Radish and Ginger on the Gram Negative Bacteria that Cause Urinary Tract Infection in Balad City

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### ABSTRACT

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#### Keywords:

*bacteria, urinary tract infection, antibiotics sensitivity, radish and ginger plants*

This study aimed to investigate the inhibitory effect of aqueous and alcoholic extracts of radish and ginger on bacterial species isolated from urinary tract and auditory system infections. Prior research has shown the potential of natural plant extracts as alternatives to synthetic antibiotics. In this study, extracts were tested at concentrations of 10, 25, 50, and 100 mg/ml for each extract against six different bacterial species. Results demonstrated that the hot alcoholic extract was more efficient than the hot water extracts of the two plants, with 100 mg/ml concentration having the highest inhibition ability. In addition, the inhibitory effect increased with increasing extract concentration. The hot alcoholic extracts of both ginger and radish were found to have higher inhibitory effects against the bacterial species, with the hot alcoholic ginger extract being more effective than the radish water extract. Furthermore, the synergistic effects of the hot alcoholic and water extracts of radish and ginger were tested against all of the identified bacterial species. Finally, sensitivity testing demonstrated that the isolated bacteria showed differences in their sensitivity and resistance to different antibiotics. Overall, this study highlights the potential of natural plant extracts as a source of new antibiotics for the treatment of bacterial infections. Existing research has shown the potential of natural plant extracts as alternatives to synthetic antibiotics for the treatment of bacterial infections, including UTIs. However, there is a lack of studies investigating the inhibitory effect of specific plant extracts, such as radish and ginger, on bacterial species isolated from UTIs and auditory system infections. This study aims to address this research gap by investigating the inhibitory effect of aqueous and alcoholic extracts of radish and ginger on six different bacterial species. The importance of this research gap lies in the increasing prevalence of antibiotic resistance and the need for alternative treatments. The research motivation is to explore the potential of natural plant extracts as a source of new antibiotics for the treatment of bacterial infections.

## 1. INTRODUCTION

Urinary Tract Infections (UTIs) are common and significant health problems in both developing and developed countries, with a majority of cases being caused by bacterial pathogens, particularly among women, children, and individuals with kidney failure. It is estimated that approximately 250 million people are infected with viral tract infections each year. UTIs are a major medical issue worldwide, with Iraq reporting a staggering 23% incidence of bacterial infections, which is the highest in the world [1, 2]. UTIs predominantly affect females and are particularly concerning for children [3]. The most common cause of UTIs is *Escherichia coli*, which accounts for 90% of cases and originates from the human intestine. Other Gram-negative bacterial pathogens in the Enterobacteriaceae family, such as *Proteus* spp., *Klebsiella* spp., and *Pseudomonas* spp., as well as *Enterococcus* spp. and Gram-positive *Staphylococcus aureus* and *Staphylococcus* spp., can also cause UTIs [3].

Treatment of UTIs:

Although antibiotics have been a critical treatment option

for UTIs, their effectiveness has been compromised due to the emergence of antibiotic-resistant bacteria. This resistance occurs due to the random increase in the use of antibiotics and the presence of plasmids responsible for this resistance, which can transfer between bacteria [4]. As a result, there is a growing interest in exploring alternative treatments for UTIs, particularly since antibiotics can cause side effects. One such alternative is the use of medicinal plants and herbs, which have been used for centuries in traditional medicine to prevent and treat various diseases. Historical texts from civilizations such as Assyrian, Greek, Pharaonic, Indian, and Chinese describe the use of medicinal plants and herbs for various ailments [5].

The research gap in this study is the need for new antibiotics to combat bacterial infections, specifically UTIs and auditory system infections. While previous research has explored the potential of natural plant extracts as alternatives to synthetic antibiotics, there is a lack of research investigating the inhibitory effects of radish and ginger extracts on bacterial species commonly found in these types of infections. The specific research gap addressed by this study is the need to evaluate the inhibitory effect of aqueous and alcoholic extracts

of radish and ginger against bacterial species isolated from UTIs and auditory system infections. The study also aims to determine the synergistic effects of the extracts and their potential as alternatives to antibiotics that are losing their effectiveness due to bacterial resistance [6].

To achieve these goals, the paper is structured as follows: first, a literature review is presented, outlining the previous research on the use of medicinal plants and herbs in the treatment of bacterial infections. Next, the methodology section details the procedures used to isolate the bacterial species and prepare the plant extracts, as well as the methods used to test their inhibitory effects. The results section presents the findings of the study, including the inhibitory effects of the plant extracts and their synergistic effects. Finally, the discussion and conclusion sections provide an interpretation of the results and their implications for future research in this area.

## 2. MATERIALS AND METHODS

### 2.1 Samples collection

This study was conducted in city Balad, hospital Balad and hospital microbiology laboratory. 100 urine samples were collected randomly from patients who attended to a Balad hospital and from this number 40 positive urine samples were contained a bacterial growth, and the rest 60 samples were administered that they were negative for bacterial growth after taking information from patients who attend the hospital and excluding patients who have used antibiotics for treatment. Samples were collected before to the use of antibiotics, and information recording age, gender, diabetes, and the presence of previous UTIs was recorded for each patient individually.

### 2.2 Diagnose bacterial isolates

The intestinal family bacteria colonies were identified by depended on the culture characteristics of the growing colonies on the medium of Mac agar, the medium of blood agar and the medium of Mannitol agar, and through the biochemical and morphological tests, and they were implanted on the medium of the Nutrient agar to complete the rest of the confirmatory tests. Some tests have been made on it, such as Oxidase detector and Catalase detector, KOvac's detector, Methyl red detector, Voges-Proskauer detector, and Citrate utilization test [7]. Samples were taken after the diagnosis and kept in the refrigerator at a temperature of 4°C for several weeks. As for keeping the isolates for a longer period, the isolates were planted after making sure of their purity to the agar slope of the nutritious agar and they were kept for a month in the refrigerator and kept for a longer period, Nutrient broth medium was prepared with added 15% sterile glycerol and inoculated by skimming a quantity of the bacterial culture on a solid medium and kept in the freezer at -20°C [8, 9].

### 2.3 Antibiotic sensitivity test

The standard Kirby-Bauer Disk method, according to studies [10, 11] was used to test the susceptibility of bacteria to antibiotics. The sensitivity of intestinal bacteria isolates developing on Muller-Hinton medium prepared from Oxoid was tested for (9) types of the antibiotics included. Chloramphenicol 30mg, penicillin 10mg, Ampicillin 10mg, Tetracycline 30mg, Gentamycin 10mg, Nalidixic acid 30mg, Vancomycin 30mg. Most of these antibiotics belong to the

pharmaceutical company Bioanalyse, where the diameters of the inhibition areas were measured and recorded. The sensitivity of the isolates to the used antibiotics was classified as sensitive (S), medium sensitivity (I) or resistant (R).

## 2.4 Plant extraction method

### 2.4.1 Method of aqueous extraction

Radish and ginger plants were obtained from the local market, where the roots of large, medium and full size were selected, with soft and healthy skin, where they were cleaned well from the dirt, if any, by washing them with tap water with confirmation on removing unwanted parts. The roots were weighed directly and cut into small parts. So, then boiled distilled water was added to it on the basis of weight/ volume, meaning that we took 1,000 grams of the roots and added a liter of water to it, then put it in the electric mixer.

The fresh raw juice was obtained after filtering the mixture by many layers of clean medical gauze, then the filtrate was centrifuged using a central centrifuge to obtain the clear filter without impurities. After that, the scent was distributed on Petri dishes and the weight of the dishes was taken into account before applying the extract and placed in the incubator at a degree Heat of 37°C for 48 hours to obtain a dry powder for the extract, and then kept in the refrigerator at a temperature of 4°C until use [12].

### 2.4.2 Method of alcohol extraction

The steps for preparing the warm alcoholic extract were followed in the same paragraph above, except for the use of ethyl alcohol at a concentration of (75%) instead of distilled water to obtain a dry plant alcoholic extract [12].

## 3. RESULTS AND DISCUSSION

100 urine samples were collected for patients with acute and chronic urinary tract infection, 40 infected samples were obtained, and the ages of the infected ranged between (10-60) years and for both gender, where the infection in females was higher than males as shown in Table 1.

As 100 bacterial isolates were obtained, it was distributed between males and females according to ages, where it was 25 cases in females 55.5%, and 15 isolates in males with ratio 27.3%, where there was a significant difference between males and females, as shown in Table 2 whereas 60 sample uninfected.

The results in Figure 1 show that *E. coli* bacteria were the most isolated with 30% and *P. aeruginosa*, *K.pneumoniae*, *S.marcescens* and *P. mirabilis* with isolation rates (13%, 20%, 12%, 15%) respectively, while we find that *C. freundii* bacteria occupies the last place of isolation rates, as it is isolated by 10%.

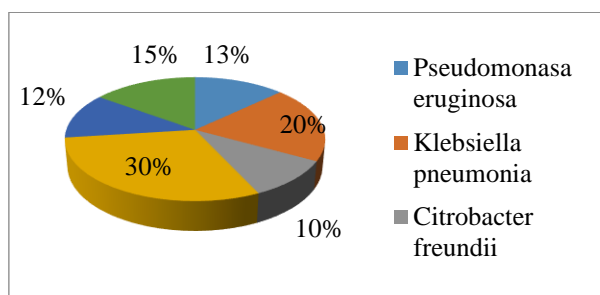
**Table 1.** The relationship between bacterial infection and gens

Bacterial infection	Gender	
	Male no. (%)	Female no. (%)
Infections	15 (27.3%)	25 (55.5%)
Non-infections	40 (72.7%)	20 (44.5%)
Total	55 (100%)	45 (100%)
Chi-Square=12.212		
<i>p</i> value=0.0003		

**Table 2.** Number of bacterial infections according to age groups

Age (year)	Gender	
	Male no. isolates (%)	Female no. isolates (%)
10-19	1 (6.67%)	2 (8%)
20-29	2 (13.34%)	8 (32%)
30-39	4 (26.66%)	5 (20%)
40-49	3 (20.00%)	4 (16%)
50-60	5 (33.33%)	6 (24%)
Total	15 (100%)	25 (100%)

Chi-Square=32.502  
p value=0.0006



**Figure 1.** Bacterial isolates

The results in Table 3 showed the numbers and percentages of susceptible isolates and antibiotic resistance included in the study by the method of diffusion in pits. The current study showed that there are isolates that bear the characteristic of multiple antibiotic resistance, and the variation in the sensitivity of bacterial isolates to antibiotics may be due to several factors that may affect the results of the examination including, the thickness of the culture media, the pH of the culture medium, the size of the vaccine used, the content of the antibiotic tablet, age Antagonist content and antagonist disc storage method, in which sensitivity to bacterial isolates was compared with what was reported by the study [13].

Through Table 3 it was found that the reason for the emergence of isolates of *P. mirabilis* bacteria resistant to many

of the antibiotics used in this study to the presence of mutations or the presence of plasmids that bear the resistance characteristics of many antibiotics combined, which play an important role in the spread of the phenomenon. Multiple antibiotic resistance is highly common among bacteria, in addition to this bacterial type having the ability to form biofilm that plays an important role in antibiotic resistance [14]. While the cause of *K.pneumoniae* resistance to many antibiotics is due to the possession of this preservative bacterium, there are also many mechanisms that make it resistant to antibiotics, and the most important of these mechanisms is its production of beta-lactase enzymes, as studies have shown that it has many types of these enzymes that destroy a wide range of antibiotics Beta-lactam [15].

Studies have indicated that the cause of resistance of *S. marcescens* bacteria may be due to the containment of the outer membrane of its strains on a large amount of fats and stable fats, as well as the containment of its strains on the resistance plasmids. As the resistance of these bacteria to antibiotics contributes to the increase in the incidence of hospital infection due to their possession of the plasmids that encode them (R-Plasmids) and resistance factors (R-Factors), as they have been shown to transmit the resistance characteristic and produce enzymes (beta-Lactamase) in particular, and it has been diagnosed. More than one type of broad-spectrum beta-lactamase is in isolation of one of them, and conjugation is considered one of the methods for transmitting the resistance trait.

Also, the high resistance shown by isolates of *Ps. aeruginosa* for most of the antibiotics used in the current study is due to the fact that these bacteria possess many antibiotic resistance mechanisms, and among these mechanisms the outer wall contains a special protein that is characterized by its high ability to release various antibiotics into the outside of the bacterial cell with the same speed of entry and this thing makes the concentration of the antibiotic Inside the bacterial cell is insufficient to eliminate it, and the reason for its resistance to antibiotics may be due to the presence of the resistance plasmid R-plasmid, which gives it the characteristic of resistance to many antibiotics [16].

**Table 3.** Sensitivity of bacterial species to antibiotics in patients with urinary tract infection

Germs types	Sensitive	Penicillin		Vancomycin		Nalidixic acid		Ampicillin		Gentimycin		Chloramphenicol	
		%	No	%	No	%	No	%	No	%	No	%	No
<i>E. coli</i>	S	16.6	5	-	-	60	18	10	3	46.7	14	33.3	10
	Ms	16.6	5	-	-	6.7	2	16.6	5	33.3	10	10	3
	R	66.8	20	100	30	33.3	10	73.4	22	20	6	56.7	17
<i>P. mirabilis</i>	S	70	7	-	-	100	10	100	10	80	8	70	7
	Ms	20	2	10	1	-	-	-	-	10	1	10	1
	R	10	1	90	9	-	-	-	-	10	1	20	2
<i>K. pneumoniae</i>	S	-	-	20	3	80	12	-	-	26.7	4	80	12
	Ms	-	-	-	-	20	3	13.4	2	40	6	6.6	1
	R	100	15	80	12	-	-	86.6	13	33.3	5	13.4	2
<i>Pseudo. aeruginosa</i>	S	-	-	-	-	-	-	-	-	12.5	1	-	-
	Ms	-	-	-	-	-	-	-	-	-	-	-	-
	R	100	8	100	8	100	8	100	8	87.5	7	100	8
<i>Citro. freundii</i>	S	-	-	83.4	5	16.6	1	-	-	33.2	2	66.8	4
	Ms	-	-	-	-	16.6	1	-	-	-	-	16.6	1
	R	100	6	16.6	1	66.8	4	100	6	66.8	4	16.6	1
<i>Serr. marcescens</i>	S	-	-	-	-	100	4	75	3	75	3	100	4
	Ms	-	-	-	-	-	-	-	-	-	-	-	-
	R	100	4	100	4	-	-	25	1	25	1	-	-

S: Sensitive, I: Intermediate, and R: Resistance

**Table 4.** Inhibitor diameter

Treatments	Concentration mg / mL	<i>Citrobacter freundii</i>	<i>Pseudo. Aeruginosa</i>	<i>K pneumonia</i>	<i>P. mirabilis</i>	<i>E. coli</i>	<i>Serratia marcescens</i>
Alcoholic Radish Extract	10	13	/	/	/	/	4
	25	16	8	12	14	7	18
	50	20	10	15	16	18	19
	100	27	14	19	20	20	22
Watery Radish Extract	10	/	/	/	/	/	2
	25	10	5	/	4	5	10
	50	11	6	7	10	8	18
	100	13	12	15	25	21	21
alcoholic Ginger Extract	10	/	/	/	2	/	8
	25	14	11	5	3	20	19
	50	21	14	22	8	21	22
	100	25	20	27	24	23	25
Watery Ginger Extract	10	/	/	/	/	/	8
	25	/	5	/	5	13	18
	50	19	8	9	18	18	18
	100	23	16	18	21	20	21

Table 4 showed the effectiveness of plant extracts on bacterial isolates isolated from the urinary tract, where the effectiveness of plant extracts was tested by the method of diffusion from pits according to what was mentioned in the materials and methods of work by testing all the diagnosed bacterial isolates under the study where it showed different efficacy towards plant extracts and concentrations (100, 50 and 25.10 mg / ml).

Table 4 shows rate inhibitor diameter and selected (3) bacterial isolate from all type, which excel resistant for large antibiotic to conduct experiment hot extraction them and record inhibition rate.

The mechanisms of inhibiting plant extracts towards bacteria can be explained, according to one of the researchers, by inhibiting the formation of the cell wall of the microorganism or by inhibiting the synthesis of some basic proteins in it and the formation of complexes with the cell wall that impede the regularity of permeability, and inhibit some enzymes that have an important metabolic role in growth and reproduction, and the rupture of cellular membranes [17].

The study of the effect of the (hot) aqueous and alcoholic plant extracts of the fruits of the Zingiber plant on the bacterial species isolated from urinary tract infections was conducted by the method of spreading from pits, which is characterized by its ease and efficiency [18]. Where 3 bacterial isolates of each type were selected and characterized by their great resistance to antibiotics to conduct the extracts experiment on them, and then recorded their inhibition rate and it was measured in units (mm). One of the studies indicated that the ginger plant consists of different components according to the type of solvent, as it showed that the aqueous extract of ginger contains flavonoids, amino acids, carbohydrates and glycosides with the absence of both sterols, tannins, phenols, terpenes, alkaloids, lecnins and soaps, while the alcoholic extract of ginger has indicated that it contains the same study. Flavonoids, amino acids, carbohydrates, alkaloids, and glycosides, with low amounts of phenols and tannins, and the absence of terpenes, lignins, and saponins.

The effect of (hot) aqueous and alcoholic plant extracts of the radish plant Raphanus on the bacterial species isolated from urinary tract infections was studied by the method of spreading from pits, which is characterized by its ease and efficiency. Where it selected (3) bacterial isolates of each type,

which was characterized by its great resistance to antibiotic, to conduct the experimental extracts on them, then recorded the rate of inhibition for them and it was measured in units (mm). Our current study showed that this plant extract has a differential effect on bacterial isolates, and this depends on the type of extract, the amount of concentration used and the type of microorganism.

Radish is an annual plant that has tuberous, wedge-shaped or spherical roots depending on the variety, and the medicinal part in it (seeds, leaves, and roots), and the active ingredients in radish are starch, sugar, Raphaiol, Raphanin, Reticol and Sinapine, the compound Raphaiol is from Alkaloids, which are the most important medicinal substances in plants, and from the chemical view are complex organic chemicals, and the roots and leaves of radish have an effective effect on the body's resistance to bacteria [19-24].

In conclusion, the study found that bacterial isolates from urinary tract infections showed various degrees of resistance to antibiotics, with some exhibiting multi-drug resistance. The emergence of antibiotic resistance was attributed to several factors, including the thickness and pH of the culture media, size of the vaccine used, age of the antagonist content, and antagonist disc storage method. Additionally, bacterial strains had inherent mechanisms for antibiotic resistance, such as the possession of plasmids or beta-lactase enzymes.

The study also found that plant extracts from ginger and radish showed varying degrees of effectiveness against the bacterial isolates, with an inhibitory effect depending on the type of extract and the concentration used. The extracts' inhibitory mechanisms may be due to the inhibition of cell wall formation, the synthesis of basic proteins, the formation of complexes with the cell wall, inhibition of enzymes, or rupture of cellular membranes. The study's findings are rich in the understanding of bacterial resistance mechanisms and the potential for plant extracts as alternative treatments for urinary tract infections.

Compared to previous studies, this study's contribution lies in its focus on urinary tract infections and its comprehensive evaluation of bacterial isolates' resistance to antibiotics and plant extracts' effectiveness. The study's findings can aid in developing better strategies for the treatment of urinary tract infections and in reducing the incidence of antibiotic resistance.

#### 4. CONCLUSIONS

**Bacterial Resistance:** The study found that bacterial isolates from urinary tract infections showed various degrees of resistance to antibiotics. Some strains even demonstrated multi-drug resistance. The resistance was attributed to multiple factors including the thickness and pH of the culture media, size of the vaccine used, age of the antagonist content, and antagonist disc storage method. Moreover, bacterial strains had inherent mechanisms for antibiotic resistance, such as possession of plasmids or beta-lactamase enzymes.

**Effectiveness of Plant Extracts:** The study found that plant extracts from ginger and radish showed varying degrees of effectiveness against the bacterial isolates, with the inhibitory effect depending on the type of extract and the concentration used.

**Mechanism of Action of Plant Extracts:** The inhibitory mechanisms of the plant extracts might be due to inhibition of cell wall formation, synthesis of basic proteins, the formation of complexes with the cell wall, inhibition of enzymes or rupture of cellular membranes.

#### 5. RECOMMENDATIONS

**Further Research:** More research should be conducted on the potential of plant extracts, especially ginger and radish, as alternative treatments for urinary tract infections. This is due to their demonstrated effectiveness against bacterial isolates.

**Reducing Antibiotic Resistance:** The findings of the study can aid in developing better strategies for the treatment of urinary tract infections and in reducing the incidence of antibiotic resistance. Thus, efforts should be made to implement these findings in clinical practices.

**Education and Awareness:** There is a need for increased education and awareness about the potential risks of antibiotic resistance and the benefits of alternative treatments, such as plant extracts.

**Regulation and Policies:** Policy makers should consider these findings when developing guidelines and regulations for the treatment of urinary tract infections. This could help ensure more effective treatment strategies and better patient outcomes.

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