

Effects of Cold and Hot Water Extracts of Onion and Ginger on Multidrug Resistant Bacteria Isolated From Urinary Tract Infection

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Abstract

Background: An alarming increase in bacterial strains resistant to existing multidrug agents demands a renewed effort to seek agents from alternative system of medicines. In this study, screening of herbal plants like bulbs of red onion and root of *ginger* was carried out to check the antibacterial ability against multidrug resistant pathogen such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* which isolate from Urine samples and

Objective: To study antibacterial activity of medical plant such as *allium cepa* and ginger to treatment most important infection in the world that is urinary tract infection with fewer side effects

Materials and Methods: A prospective study was conducted on 100 UTI infection patients attended the Baquba hospital to Diagnosis urinary tract infection for period between May 2013 to October 2013, the sample take from two gender male and female after that the bacterial isolation take place base on Bergey, secondly collect of herbal plant and preparation of extraction and finally antimicrobial screening done by disc diffusion method.

Results: The results showed there were pathogenic bacteria isolated from urine samples is high in the case of female which were 76 cases infected with pathogenic bacteria more than in male which were 24 cases infected with same pathogenic bacteria ,two types of bacteria had resistant present also in samples called multi drug resistant included staphylococcus and pseudomonas ,second things the results showed also that the cold-water extract of ginger affected both organisms at all concentration at p value <0.01 same things in the hot water of ginger extract but the different the hot water extract affected only *pseudomonas aeruginosa* and there is no affected on staphylococcus aureus

Conclusion: The effect of ginger extracts to treat multi drug resistant bacteria more significant than extracts of onions at p<0.05.

Key words: Multidrug resistant, MRSA, *allium cepa*, *zingiber officinale*

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Introduction

One of the most common infections in the world is urinary tract infection which is more common in woman than man, in the united states it account for nearly 8 million patients and 1.5 million attempt hospital [1, 2, 3, 4].

Plants in nature has been a source of making medicinal agents by different methods for thousands of years to treat a variety of diseases and huge number of modern drugs have been made from natural sources. Plant- derived drugs remain an important resource, especially in developing countries, to combat serious disease. Approximately 60–80% of the world's population still relies on traditional medicines for the treatment of common illness. It is believed that the history of herbal medicine began with the earliest man, the first written herbal record was in 2800 BC and herbal medicine is practiced today in countries around the world [5]. Some medical benefit of plant over the formulated drugs are that they typically have fewer side effects and may be less safe to use over time, they are inexpensive compared to formulated drugs and they are readily available [6].

The most important medical plant in history of herbal medicine is bulb Onion (*Allium cepa*). Onion is one of common local vegetables which have been used as source for food and medicine. The plant is in tree types according to the skin; red, white and purple which is used as source for energy [7, 8]. The extract of Onions has shown effective against common cold, heart disease, diabetes, osteoporosis, coughs a sore throat [9]. Researches approve their effect to inhibit bacterial grow (bacteristatic) [10]. Certain chemical compounds believed to have anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties such as quercetin are present in onions [11]. They are high in flavonoids which is concentrated on the outer

layer of the flesh [12]. Onions are also high in polyphenols than other allium vegetables [13].

Other important herbal plant that is Ginger is the rhizome of the plant *Zingiber officinale*. It is a tropical species native to South East Asia, the top five country cultivate ginger are India, Thailand, China, Nepal, and Nigeria. For over 5000 years ginger used in Asian in medicine to treat stomach aches, nausea, and diarrhea. Many digestive, anti-nausea, and cold and flu dietary supplements sold in the United States contain ginger extract as an ingredient [14, 15]. Gingerols present in ginger have analgesic, sedative, antipyretic, antibacterial and gastrointestinal tract motility effects [13]. The rapid development of multidrug resistant bacteria has become threatens the effective prevention and treatment of infections caused by bacteria that requires action across all government sectors and society [16, 17, 18, 19, 20, 21].

This study was done to analysis the number of patients had infections of UTI which are woman or man more infected with UTI and treat this infection by used extracts of herbal plants (as alternative drugs) and finally to investigate the beneficial effect of 2 extracts like onion and ginger to effect on multi drug resistant bacteria causes these infection.

Materials and Methods

Microbial Strains

The *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated in microbiology laboratory at College of Medicine - Diyala University, hundred urine samples collect from Baquba teaching hospital. Clinical strains were preserved on nutrient agar. Colony characteristics and bacterial morphology were studied and diagnosis confirmed by gram stain, Coagulase test and Biochemical tests

(catalase, oxidase, nitrite reductase, Methyl red, voxproskawer test, Indole production, Urease, Hydrogen sulfide Production and Citrate utilization)[17]. Sensitivity test carry out to isolate only strain that resistance to antibiotic by Kirby baur disc method.

Collection of Plants:

Bulbs of red onion and root of ginger were chosen for present study, these plants were collected from market in Baquba city.

Preparation of the Extracts of the Plant Materials:

Fresh onion bulbs and ginger were carefully dressed kept in refrigerator at 20° C until used.

A- Onion Extraction:

Fresh bulbs of onion were manually washed using sterile distilled water then extracted in the following steps, the cold water extraction done by exactly 100 g of Onion bulbs were cursed in a homogenizer to small pieces then added 50 ml of sterile cold distilled water for one day in laboratory shaker device for proper extraction. The Mixer was filtered through filter paper in a flask and kept in sterile flask in refrigerator at 20°C until to be used for checking antimicrobial activity. Hot water extraction done by exactly 100 g of onion bulbs were cursed in a homogenizer to small pieces then added 50 ml of sterile hot distilled water for one day in laboratory shaker device for proper extraction. The mixture was filtered through filter paper and kept in a sterile flask in refrigerator at 20°C until to be used for checking antimicrobial activity [3].

B- Ginger Extraction

Scrub the root of ginger under tap water with a stiff brush to remove surrounding soil and washed with sterile Distilled Water. Then peeling ginger root manually and washed then extracted in the following steps; 100 gm of ginger root were cursed in a homogenizer to small pieces and added 50 ml of cold distilled water for one day in laboratory shaker device for proper

extraction. The mixture was filtered through filter paper and kept in sterile flask at 20°C until to be used for checking antimicrobial activity, 100 gm of ginger root were cursed in a homogenizer to small pieces and added 50 ml of Hot Distilled Water for one day in laboratory shaker device for proper extraction. The mixture was filtered through filter paper and kept in sterile flask at 20°C until to be used for checking antimicrobial activity [18].

Preparation of Inoculums

The bacterial inoculums prepared by direct colony suspension method to get density of bacteria 1×10^8 cfu. In which a small volume of sterile water was poured inside a test tube to which general colonies of the test organisms, taken directly from the plate were emulsified and the suspension was adjusted to match the 0.5 McFarland's standard which has a similar appearance of an overnight broth culture by adding distilled water [19].

Antibacterial Analysis:

The antibiotic susceptibility test was done by determining MIC value using broth dilution method and using Kirby-Bauer disk diffusion method Antibiotics used were amoxicillin (25µg), Augumentin (30µg), co-trimoxazole (25µg), gentamicin (10µg), nalidixic acid (30µg), nitrofurantoin (300µg), ofloxacin (30µg), and tetracycline (30µg), After 24/48 hours of incubation at 37°C, the diameter of the zone of inhibition was measured around each antimicrobial disk on the plate. On the basis of zone size around each antimicrobial disk results were interpreted as sensitive, intermediate or resistant according to current NCCLS standards in accordance with WHO requirements [20].

Antimicrobial Screening Test

Antimicrobial activity of the extracts of onions and ginger were evaluated against two bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). Plants were

collected and the hot and cold extracts were obtained by standard methods. Agar well diffusion method was used to test the antimicrobial activity [20]. One loopful of the microorganism was inoculated into 50ml of nutrient broth. The flasks were incubated overnight on shaker at 100 rpm. After incubation, the bacterial culture was diluted and spread on Mueller Hinton agar plates by streaking the bacterial culture on entire of the medium with the help of sterile disposable swab. Preparation of different concentrations of plant extract by made disc from filter paper and put it in premade suspension of the Onion and Ginger extracts prepared in the following order (0.1gml^{-1} , 0.2gml^{-1} , 0.4gml^{-1} , 0.6gml^{-1} and 0.8gml^{-1}). Exactly 0.5gml^{-1} of tetracycline used as a positive control. All plates then incubated at 37°C for 24 Hours.

Results were recorded as presence or absence of zone of inhibition .The inhibitory zone indicated absence of bacterial growth and it was reported as positive (growth inhibition observed) and absence of zone as negative. The test was repeated once again to insure reliability of the result [20].

Statistical Analysis of results

Data analysis was done by using t-test, ($p < 0.05$) considered significant [21].

Results

Screening samples in Baquba hospital show highly number in some pathogenic bacteria like *Staphylococcus aureus* whereas low number in other cases include *Klebsiella*, *Proteus* and *Pseudomonas spp.* as identified by using Macroscopically and Microscopically criteria and biochemical tests as shown in Figure (1).

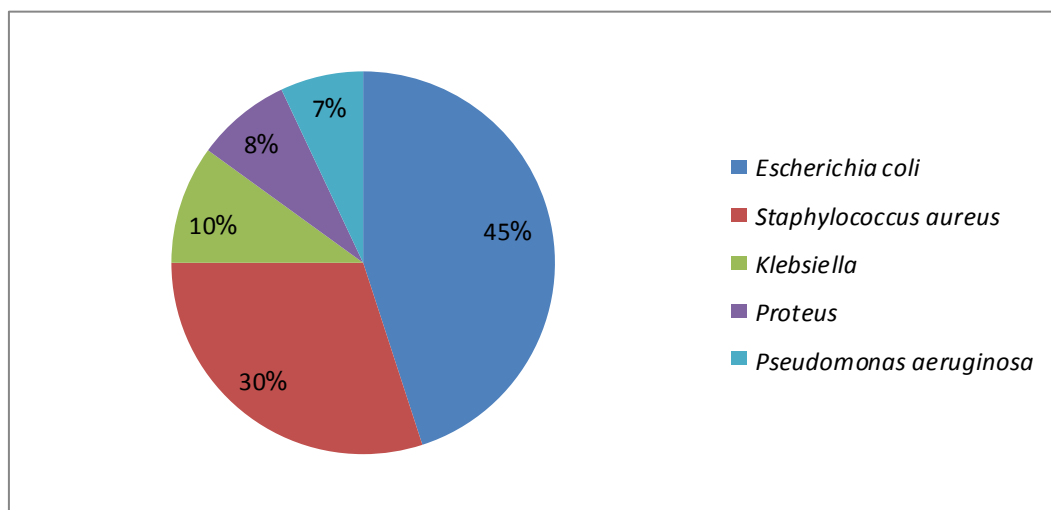


Figure (1): screening numbers of pathogenic bacteria urine sample.

According to analysis of urine sample and as shown in figure (2), 77 samples show mix of bacteria while 23% show pure bacteria.

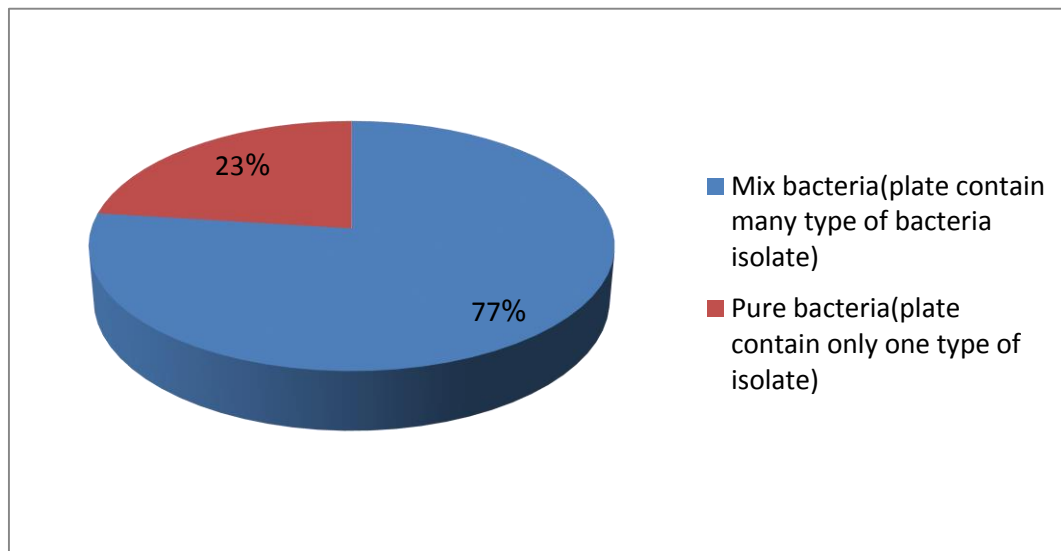


Figure (2): Analysis of urine sample.

Figure (3) show the number of cases had pathogenic bacteria isolated from urine samples is high in the case of females which

were 76 case infected with pathogenic bacteria more than in males which were 24 case This due to many reasons includes.

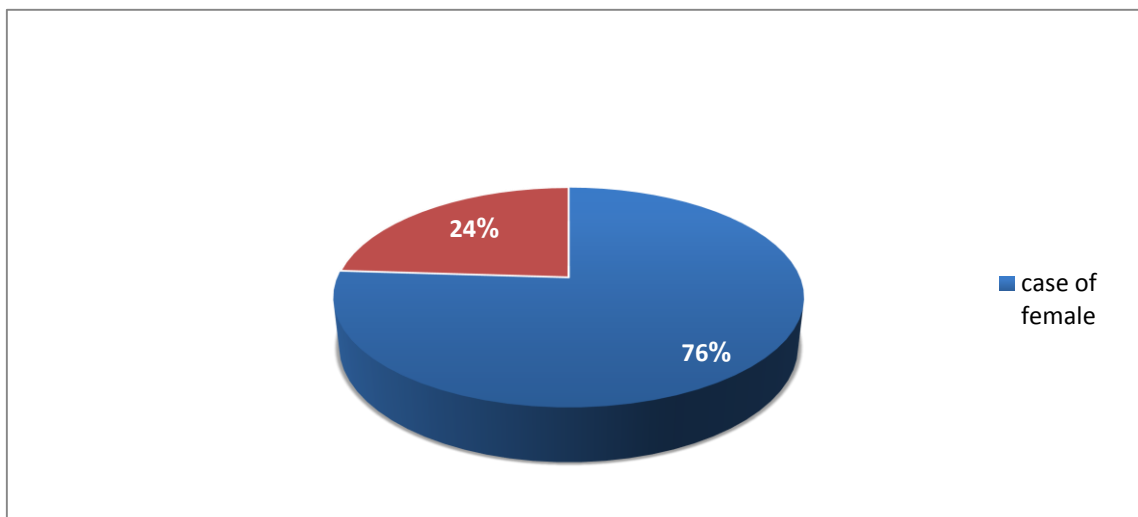


Figure (3): Number of case had pathogenic bacteria isolate from urine sample.

The *Staphylococcus aureus* show resistance to trimethoprim and methicillin antibiotics while *Pseudomonas aeruginosa* show resistance to trimethoprim antibiotic only as shown in Table (1). Therefore we can test plant extract in these two organisms because presence of multidrug resistant bacteria in hospital great concern due to the

influx of people who patronize these places in search of treatment from disease. In detail in the table 1 we can see also there is no effect of 5 antibiotics against *staphylococcus aureus* but in the case of *pseudomonas aeruginosa* there are 6 antibiotics that mean more resistance by one antibiotic in addition in the *pseudomonas* bacteria.

Table (1): Antibiotic sensitivity test for *Staphylococcus aureus* and *Pseudomonasaeruginosa*.

Antibiotics	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
Ampicillin	-	-
Trimethoprim	2 mm	5 mm
Nitrofurantoin	-	-
Carbenicillin	-	-
Rifampin	-	-
Chloramphenicol	-	-
Methicillin	20 mm	-

As can be seen in figures (4) the cold-water extract of onion show antimicrobial activity against *Pseudomonas aeruginosa* at concentration 0.8 gml⁻¹ and 0.6 gml⁻¹. While no activity had been seen against *Staphylococcus aureus*. The factors responsible for this high susceptibility of

P. aeruginosa to the extracts are not exactly known but may be attributed to the presence of secondary plant metabolites. The relation between zone of inhibition and concentration of cold extract result was found to be significant at concentration 0.8 mg l⁻¹ (p <0.05).

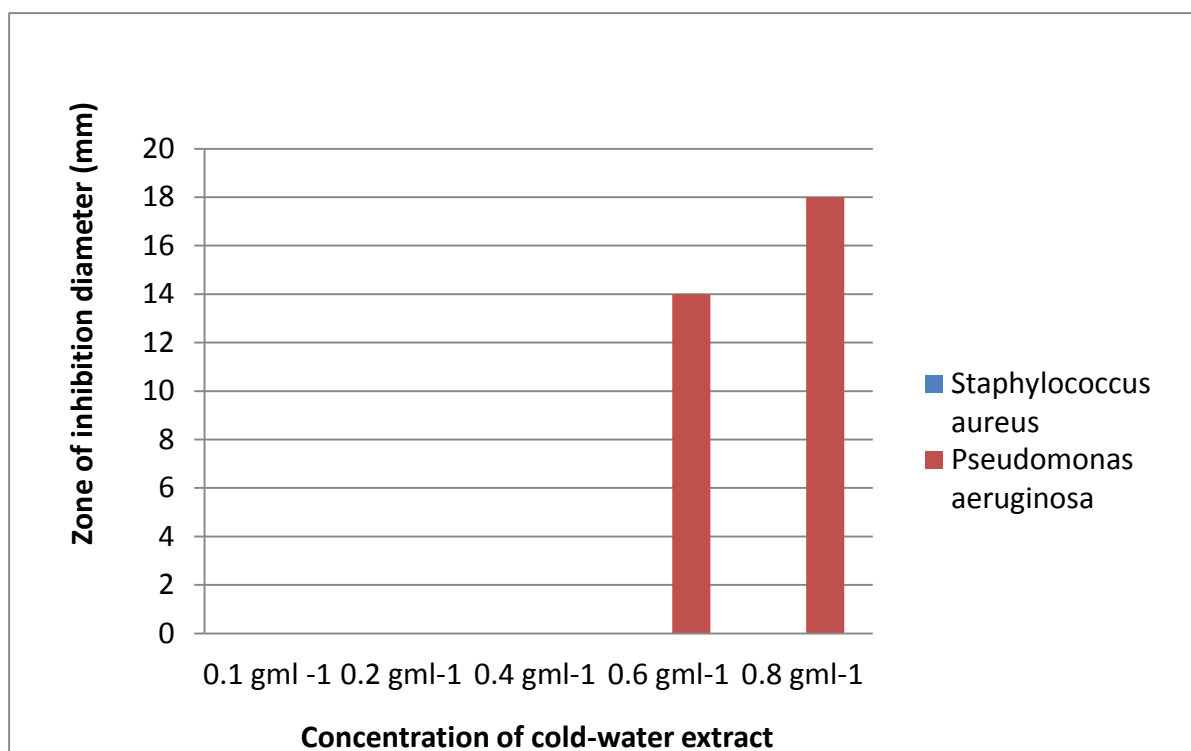


Figure (4): susceptibility pattern of cold-water extract of onion (p < 0.05).

It was observed that the Hot water extract of Onion show no activity against *Pseudomonas aeruginosa* at all concentration while the extract had activity against *Staphylococcus aureus* at concentration 0.6 gml⁻¹ and 0.8 gml⁻¹ as shown in Figure (5).

In fact, antibacterial substances in the herbal plants are affected by heat (heat-

labile), therefore the specie lost its antibacterial activities less than 30 min at 100°C. Since, the reason of antibacterial activity against *Staphylococcus aureus* is not known yet.

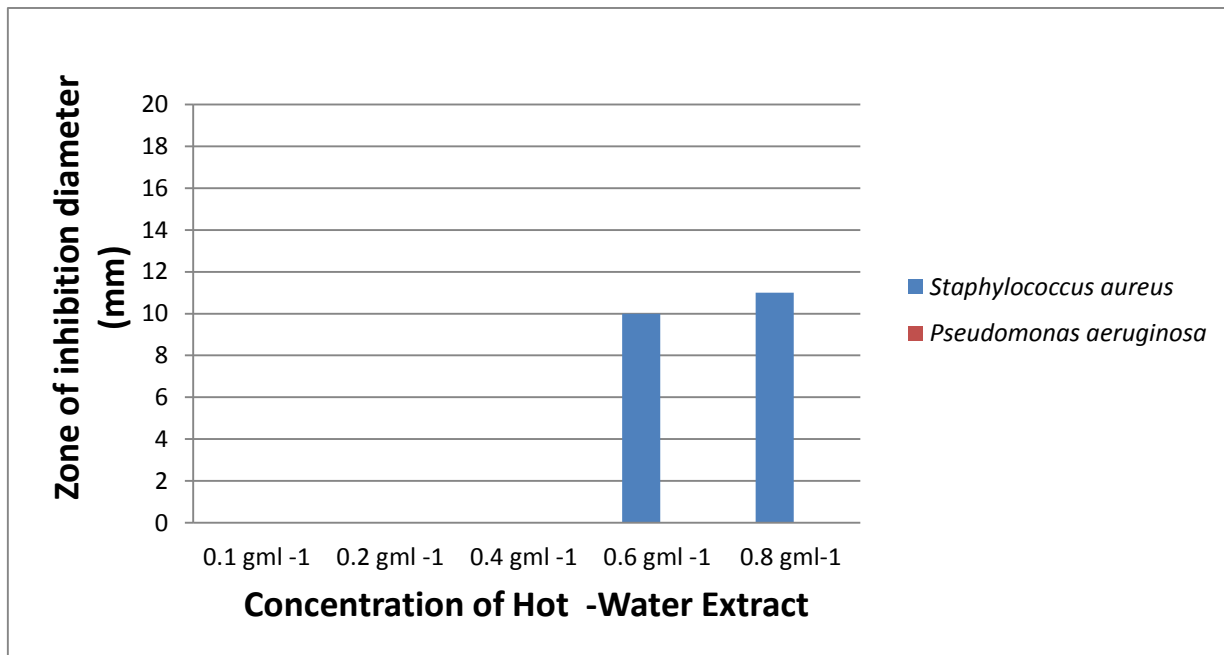


Figure (5): Susceptibility pattern of Hot -water extract of onion ($P < 0.05$).

It is clear from Figure (6) that the cold-water extract of ginger affected both organisms at all concentrations with the high

significant at ($p < 0.01$). Thus may be due to those active compounds of ginger liberating by cold water better than in onion.

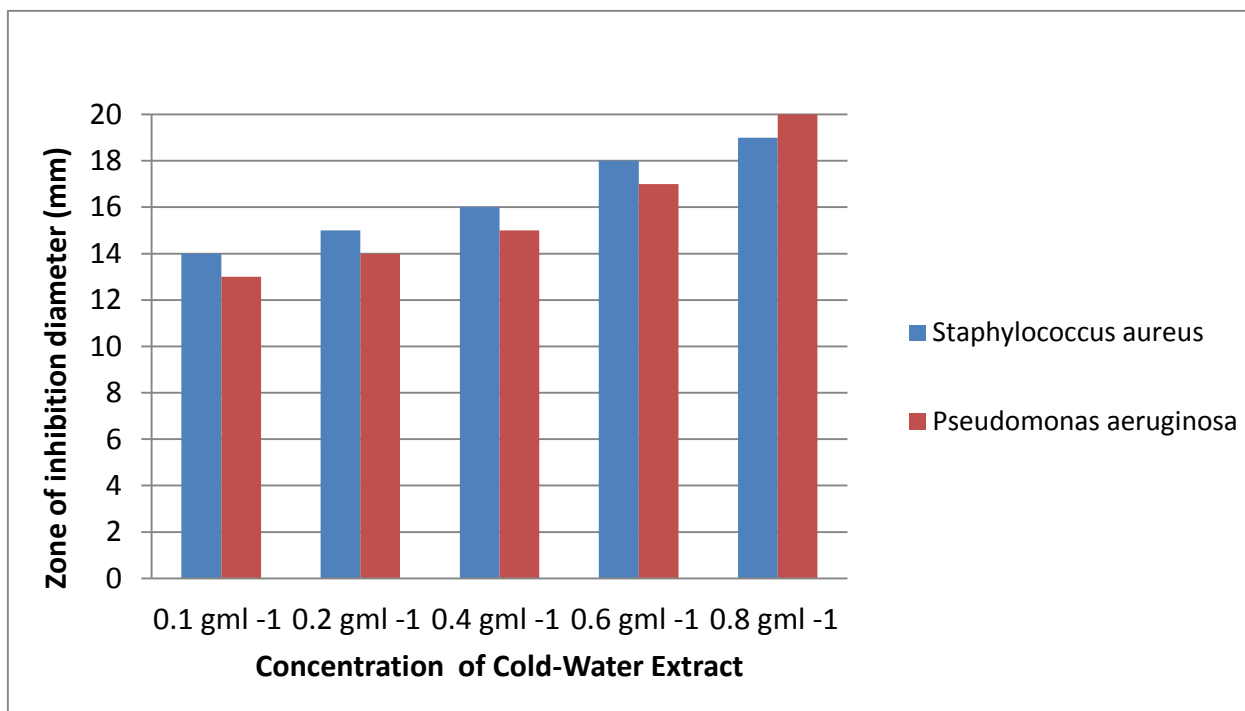


Figure (6): susceptibility pattern of Cold-water extract of ginger ($p < 0.01$).

It has been known that extracted compounds with high molecular weight take long time to diffuse, whereas extracted compounds with low molecular weight take short time to diffuse more than other compounds.

Also *Pseudomonas aeruginosa* show susceptibility against Hot Water Extract at almost all concentrations with value of high significant at ($p < 0.01$) as shown in Fig (7).

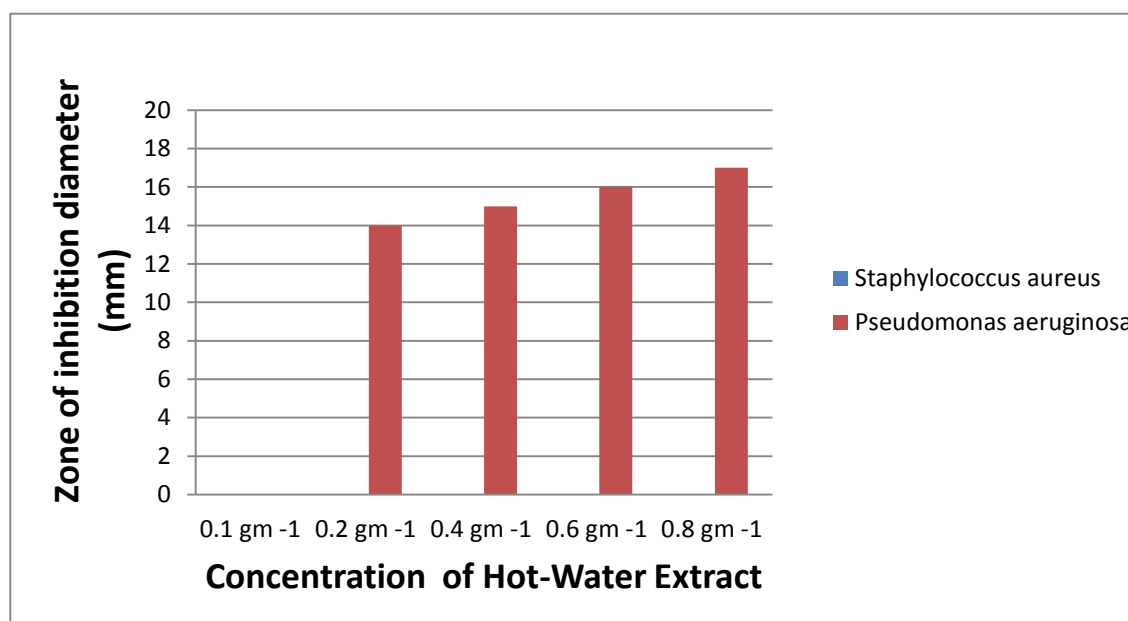


Figure (7): susceptibility pattern of Hot-water extract of ginger ($p < 0.01$).

Discussion

Urinary tracts infection present in the woman more than man because most of case it was woman show that thing in the results and Most bacterial infections of urinary tract caused by *Escherichia coli* comparing with other pathogenic bacteria [22]. *Pseudomonas aeruginosa* and *Staphylococcus aureus* which are known by their multidrug resistant ability, hence to treat these bacteria it should used alternative treatment which represented by herbal plants which is cheap and less affected side, several researches have approved their activity to control and treat diseases [23, 24].

The most common herbal plants in our local market are Red Onion and Ginger which shown antibacterial activity against both gram negative and gram positive bacteria and using extracts of herbal plants by simple methods may help treat the urinary tract infections.

Onion and Ginger are valuable plants sources of medicinally useful compound that has been traditionally used for several applications. Extract these plants by cold and hot water methods were carried in our study which it was not surprising as clear that

ginger have antibacterial power against two test pathogenic bacteria compared to antibacterial power of Onion ,Other studies conclude that a onions and ginger extracts has benefit [3].

The differences in antibacterial effects of these two plants are due to phytochemical constituents, beside the sensitivity of Gram negative *Pseudomonas aeruginosa* were found to be different with regard to Gram Positive *Staphylococcus aureus* to the tested plants because of the difference in the morphology, as well as cell wall of *Pseudomonas aeruginosa* composed of two barrier layer represented by Phospholipids and Lipo-polysaccharide whereas cell wall of *Staphylococcus aureus* composed of only layer of peptidoglycan. Other study done also about onions but more pathogenic bacteria is tested [25]

In conclusion, urine sample has been found *Pseudomonas aeruginosa* is the most bacteria in the urine samples. Cold extracts of onion bulbs showed antimicrobial activity only against *Pseudomonas aeruginosa* at concentrations 0.8 gml⁻¹. The hot extract show activity at same concentration (0.8 gml⁻¹) against *Staphylococcus aureus*.

The best antimicrobial activity in our study was shown by the hot water extract and cold water extract of ginger at all concentration against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, whereas the hot extract and cold extract of onions show activity only at 0.8 gml⁻¹, 0.6 gml⁻¹ concentrations against both bacteria that mean the ginger extracts more significant than onion extracts in this experiment at p<0.01

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