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RESEARCH ARTICLE

Antibacterial activity of parsley and celery aqueous extract on the isolated bacteria from children UTI in Erbil city.

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Abstract

This study investigates the most common urinary tract infection bacteria and their sensitivity to antibiotic and some plant extraction. The urinary tract bacteria were sampled from patients of urinary tract infection (infants and children under fifteen years old) from Erbil hospital of children from 20-11-2013 to 20-1-2014. The bacteria were isolate, cultured and identified. 120 bacteria isolate were identified as *E. coli* 56 (46.66%), *Staphylococcus aureus* 19 (15.8%), *Staphylococcus sp.* and *Proteus spp.* 6 (5%) *Klebsiella sp* and *Pseudomonas aeruginosa* 5 (4.16%) each of them. *Klebsiella pneumoniae* and *Pseudomonas sp.* 4 (3.33%), *Proteus mirabilis* 3 (2.5%) *Staphylococcus albus*, *Morganella morganii* and *Micrococcus* 2 (1.66%) each of them. *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Klebsiella oxytoca*, *Citrobacter freundii* and *Pseudomonas luteola* 1 (0.83%). The isolates showed different degrees of sensitivity to different antibiotics. Antibacterial activity of watery celery extraction was ranged (2-20) mm and the Alcoholic celery extracted was ranged between (2-23) mm. watery parsley extraction (2-21) mm, alcoholic parsley extraction (2-22) mm.

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Introduction

Urinary tract infection (UTI) is one of the most common causes of hospitalization and referral to outpatient settings in children. (Al-Kareemi, 2012) The urinary tract is the body's filtering system for removing waste liquid, or urine; it comprises the kidneys, ureters, bladder and urethra (Ramadan, 2003). A urinary tract infection is caused by bacteria that enter the urinary tract; women are more likely than men to get UTI because of their urinary tract's design, (Shaaban *et al.*, 2012) Most of UTI are caused by gram-negative bacteria like *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella sp.*, *Pseudomonas aeruginosa*, *Acinetobacter*, *Serratia*, and *Morganella morganii*. Also UTI are caused by Gram positive bacteria include *Enterococcus*, *Staphylococcus*, and *Streptococcus agalacticae* (Tangho&Mcaninch, 2004).

Chemotherapeutic agent such as different antibiotics has been used for the treatment of bacteria associated UTI for several years. But, uropathogenicare increasingly becoming resistant to available antimicrobials. For this reason searching new alternative medicine to control pathogens has become a crucial part of drug development research. On the other hand, herbal medicine has been used for the medication of different bacterial disease and very few reports are available on the bacteria to resist against natural products. (Fuad., 2012).

Material and Methods:-

Sample collection:-

A total 120 urine samples were collected from 180 infants and children (below 15 years of age) infected UTI. From 20/11/2013 to 20/1/2014, from Erbil hospital for Children. The samples were collected aseptically in sterile tubes.

Diagnosis bacteria:-

All isolated on respective selective and differential media were identified on the basis of colonial, morphological, Gram stain and biochemical tests,

Biochemical tests used for identification of bacteria including: Urease, catalase, oxidase, indole, Methyl red, Voges Proskauer, Simmons citrate utilization, TSI. To the finally diagnosis used API20E. (Baron, *et al.*, 2007).

Antibiotic susceptibility test:-

Antibiotics susceptibility was carried out on all isolate using Kirby Bauer disc diffusion method. Results were interpreted by measuring the zone of inhibition in mm (Vandepitte*et al.*, 2003).

Collection plant:-

Collection plant samples both plants parsley and celery (alcoholic and watery) were obtained from market in Erbil City. The plants were washed with tap water then were washed with distilled water. They were then air dried, powdered, and stored in refrigerator at 4°C for further processes (Hero and Jwan, 2012).

Extract preparation:-

A total of 10 gm. of the plant powder was steeped in 100 ml of each solvent (ethanol and sterilized distilled water) for 3 days, and then filtered through eight-layered muslin cloth. They were filtered using filter paper (Whatman No.1) and centrifuged at 3000×g for 10 minutes. The supernatants were collected separately and stored in sterile bottles at 4°C. (Parekhetal., 2005).

Disc diffusion method used for plant extraction:-

The plant extracts were dissolved in sterile distilled water to a final concentration 50mg/ml. The disc diffusion method was used to evaluate the antibacterial activity. Mueller Hinton agar was prepared in the plates as the media for the test microorganisms. Sterile filter paper discs were impregnated with 100 µl of each of the extracts, placed on Mueller Hinton agar plate inoculated w bacteria, then incubated for 24 hr. at 37° C. Distilled water served as negative control and Impinem was used as standard to confirm that all the microorganisms tested were inhibited by the antibiotic. The antibacterial activity were evaluated by measuring the zone of growth inhibition surrounding the discs (Olaleye2007).

Results:-

A total of (120) bacterial isolate were collected from clinical sample of UTI patients from Erbil hospital for Children, 80 female (66.66%) and 40 male (33.33%) Fig. (1) The sample were obtained from patients in (infant – 15) years old. Table (1).

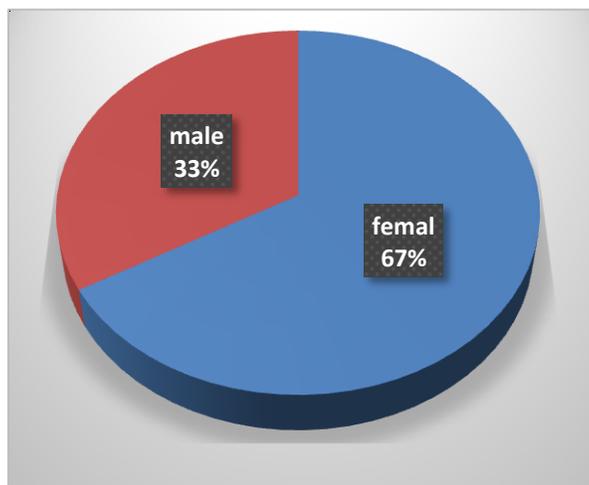


Fig. (1):- Distribution of gender in UTI patents.

Table (1) Distribution of age in UTI patents

Year	No.	%
0>-1	23	19.16
1-2	9	7.5
2-3	13	10.8
3-4	18	15
4-5	9	7.5
5-6	14	11.66
6-7	9	7.5
7-8	7	5.83
8-9	8	6.66
9-10	5	4.16
10-11	3	2.5
11-15	2	1.66
Total	120	100

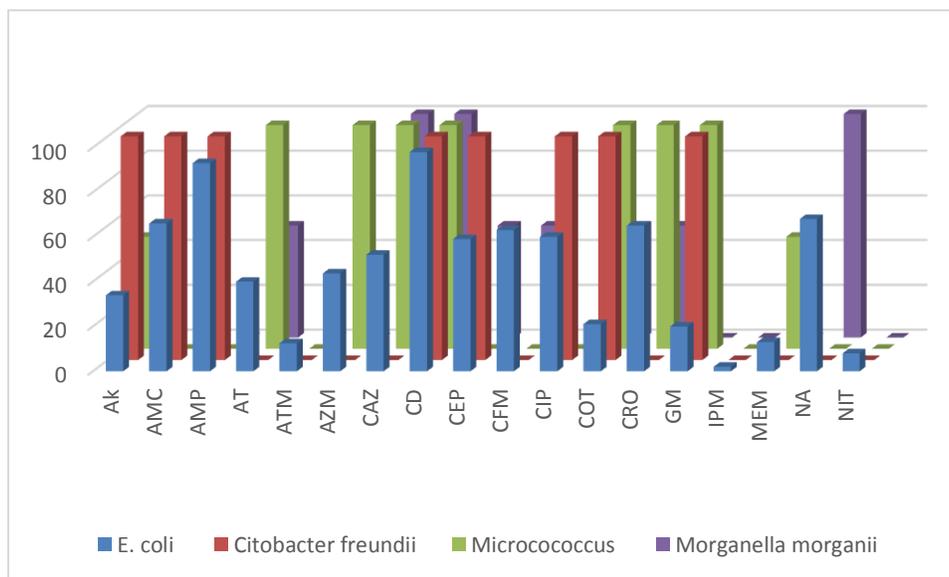
The results have shown that *E. coli* 56 (46.66%) was found to be the predominant isolates from positive urine sample of the isolates tested, *Staphylococcus aureus* 19 (15.8%), *Staphylococcus sp.* and *Proteus spp.* 6 (5%) *Klebsiella sp* and *Pseudomonas aeruginosa* 5 (4.16%) each of them. *Klebsiella pneumoniae* and *Pseudomonas sp.* 4 (3.33%), *Proteus mirabilis* 3 (2.5%) *Staphylococcus albus*, *Morganella morganii* and *Micrococcus* 2 (1.66%) each of them. *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Klebsiella oxytoca*, *Citrobacter freundii* and *Pseudomonas luteola* 1 (0.83%) Table (2).

Table (2):-percentage of bacterial isolate from urinary tract infection patients.

Bacteria isolate	No. of Isolate	%
<i>E. coli</i>	56	46.66
<i>Staphylococcus sp.</i>	6	5
<i>Staphylococcus albus</i>	2	1.66
<i>Staphylococcus capitis</i>	1	0.83
<i>Staphylococcus epidermidis</i>	1	0.83
<i>Staphylococcus aureus</i>	19	15.8
<i>Proteus sp.</i>	6	5
<i>Proteus mirabilis</i>	3	2.5
<i>Proteus vulgaris</i>	1	0.83
<i>Klebsiella sp.</i>	5	4.16
<i>Klebsiella pneumoniae</i>	4	3.33
<i>Klebsiella oxytoca</i>	1	0.83
<i>Morganella morganii</i>	2	1.66
<i>Micrococcus</i>	2	1.66
<i>Citrobacter freundii</i>	1	0.83
<i>Pseudomonas sp.</i>	4	3.33
<i>Pseudomonas aeruginosa</i>	5	4.16
<i>Pseudomonas luteola</i>	1	0.83
Total	120	100

All isolate was multi drug resistant against antibiotic. All bacteria of *E. coli*, resistance to all antibiotic and sensitive to Meropenem, Nitrofurantoin, Gentamicin, Aztreonam, and Co-trimoxazole, *Morganella morganii* resistance to all antibiotic and sensitive to Ampicillin, Aztreonam, Azithromycin, Ciprofloxacin, Gentamicin, Imipenem, Meropenem and Nitrofurantoin. *Micrococcus* resistance to all antibiotic and sensitive to Amoxicillin/clavulanic acid, Ampicillin, Aztreonam, Cephalothin, Cefixime, Ciprofloxacin, Imipenem, Nalidixic acid, Nitrofurantoin. *Citrobacter freundii* resistance to all antibiotic and sensitive to AT, Aztreonam, Azithromycin, Cefazidime, Ciprofloxacin, Imipenem, Meropenem, Nalidixic acid, Nitrofurantoin (Figure 2A). All bacteria *Staphylococcus sp.* were resistance to Amoxicillin/clavulanic acid, Ampicillin, Aztreonam, Meropenem,

Azithromycin, Ceftazidime , Cefridacin , Cyclodextrin , AT, Ciprofloxacin , Co-trimoxazole , Cefixime , Cephathiane and sensitive to Amikacin , Impinem, Gentamicin , Nalidixic acid , Nitrofuration.(Figure 2B). *Proteus sp.* showed resistance to all antibiotic only Aztreomycin , Ciprofloxacin , Meropenem (Figure 2C). *Pseudomonas sp.* Showed sensitive only to Amikacin AT, Ciprofloxacin, Gentamicin, Meropenem and Impinem (Figure 2D). *Klebsiell sp.* were resistance against all antibiotic while sensitive against Aztreomycin ,Gentamicin , Meropenem, Nalidixic acid , Nitrofuration and Impinem (Figure 2E) .



Figure(2- A) Percentage of resistance antibiotic pattern of bacteria.

AK: Amikacin , AMC: Amoxicillin / Clavulanic acid , AMP: Ampicillin , AT , ATM: Aztreomycin , AZM: Azithromycin , CAZ: Ceftazidine , CD: cyclodextrin , CEP :Cephathiane , CFM: Cefixime , CIP: Ciprofloxacin , COT: Co-trimoxazole , CRO: Cefridacim , GM: Gentanmicin, IPM: Impinem, MEM: Meropenem, NA: Nalidixic acid , NIT: Nitrofuration.

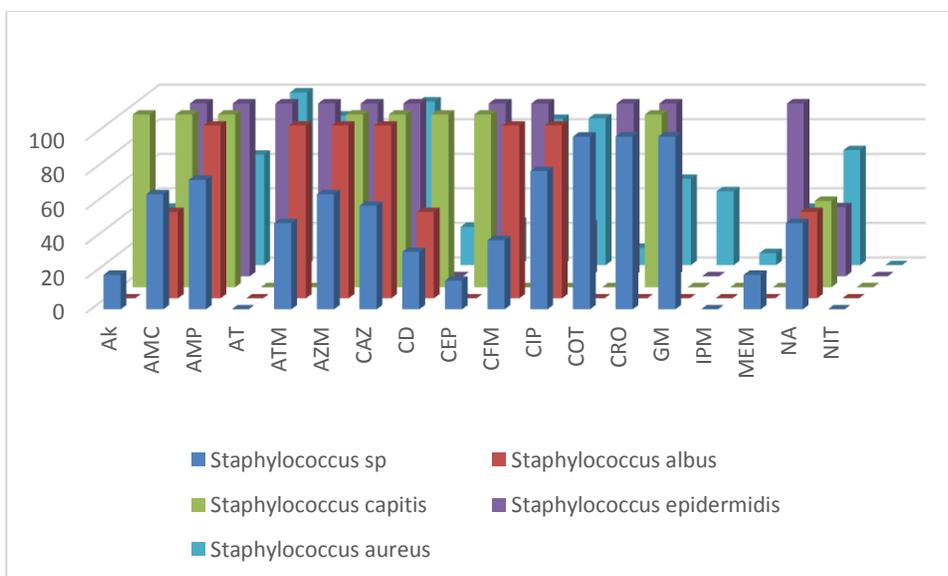


Figure (2-B) Percentage of resistance antibiotic pattern of *Staphylococcus sp.*

AK: Amikacin , AMC: Amoxicillin / Clavulanic acid , AMP: Ampicillin , AT , ATM: Aztreomycin , AZM: Azithromycin , CAZ: Ceftazidine , CD: cyclodextrin , CEP :Cephathiane , CFM: Cefixime , CIP: Ciprofloxacin , COT: Co-trimoxazole , CRO: Cefridacim , GM: Gentanmicin, IPM: Impinem, MEM: Meropenem, NA: Nalidixic acid , NIT: Nitrofuration.

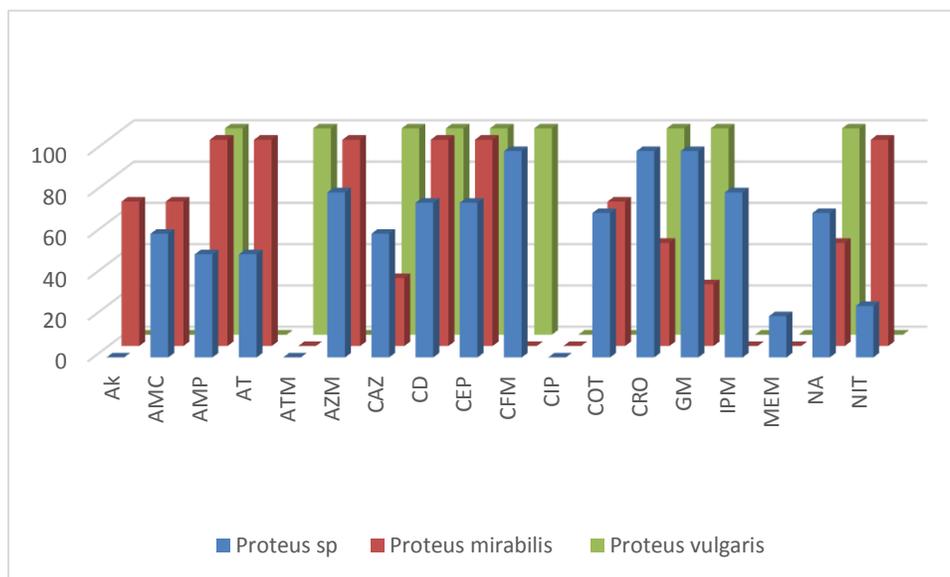
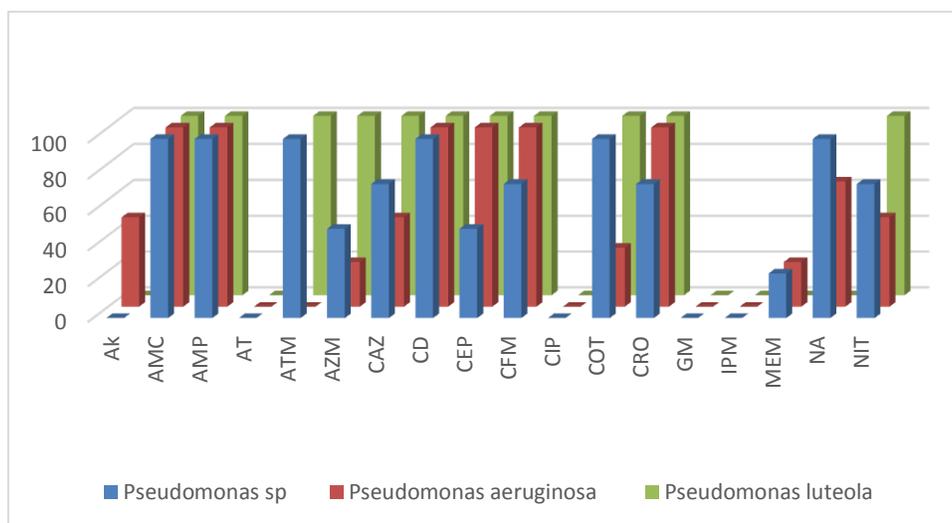


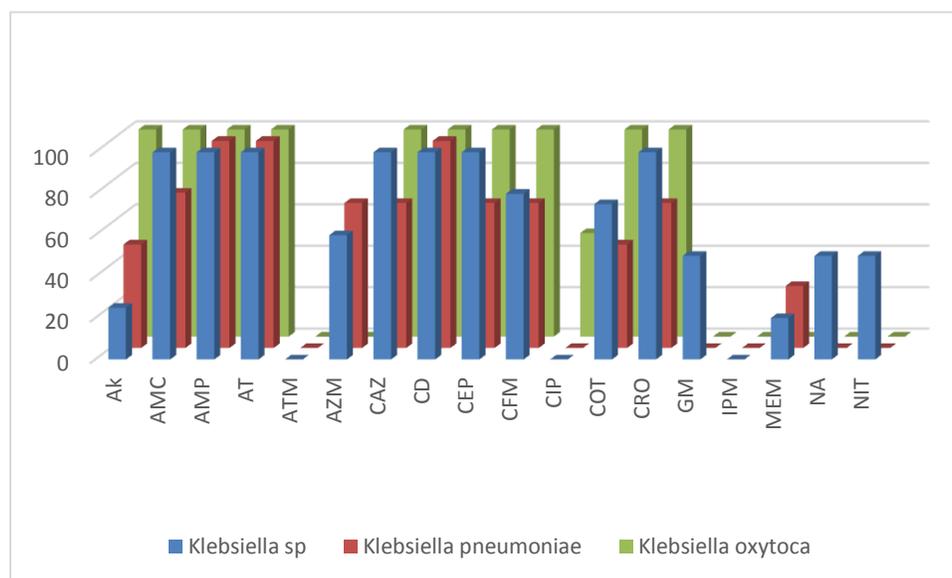
Figure (2-C) Percentage of resistance antibiotic pattern of *Proteus sp.*

AK: Amikacin , AMC: Amoxicillin / Clavulanic acid , AMP: Ampicillin , AT , ATM: Aztreomycin , AZM: Azithromycin , CAZ: Ceftazidine , CD: cyclodextrin , CEP :Cephathiane , CFM: Cefixime , CIP: Ciprofloxacin , COT: Co-trimoxazole , CRO: Cefridacim , GM: Gentanmicin, IPM: Impinem, MEM: Meropenem, NA: Nalidixic acid , NIT: Nitrofuration



Figure(2- D) Percentage of resistance antibiotic pattern of *Pseudomona* sp.

AK: Amikacin , AMC: Amoxicillin / Clavulanic acid , AMP: Ampicillin , AT , ATM: Aztreomycin , AZM: Azithromycin , CAZ: Ceftazidine , CD: cyclodextrin , CEP :Cephathiane , CFM: Cefixime , CIP: Ciprofloxacin , COT: Co-trimoxazole , CRO: Cefridacim , GM: Gentanmicin, IPM: Impinem, MEM: Meropenem, NA: Nalidixic acid , NIT: Nitrofuration.



Figure(2- E) Percentage of resistance antibiotic pattern of *Klebsiella* sp.

AK: Amikacin , AMC: Amoxicillin / Clavulanic acid , AMP: Ampicillin , AT , ATM: Aztreomycin , AZM: Azithromycin , CAZ: Ceftazidine , CD: cyclodextrin , CEP :Cephathiane , CFM: Cefixime , CIP: Ciprofloxacin , COT: Co-trimoxazole , CRO: Cefridacim , GM: Gentanmicin, IPM: Impinem, MEM: Meropenem, NA: Nalidixic acid , NIT: Nitrofuration.

The result of antibacterial activity of Watery Celery extract of all bacteria revealed that the inhibition zone (2-20) mm, while there were no effect up to *Micrococcus*. Antibacterial activity of Alcohol Celery extract of all bacteria produced inhibition zones between (2-23) mm .Table (3)

Table (3) Antibacterial activity of Celery at different concentration against different bacteria.

Bacteria isolate	Watery Celery extract Inhibition zone in (mm)					Alcoholic Celery extract Inhibition zone in (mm)					Antibiotic Impinim (Control)
	Concentration of extract										
	12.5	25	50	75	100	12.5	25	50	75	100	
<i>E. coli</i>	-	-	-	9	10	-	-	5	9	11	18
<i>Staphylococcus sp.</i>	-	-	9	10	12	2	5	12	20	21	18
<i>Staphylococcus albus</i>	-	-	5	8	13	2	4	8	11	13	20
<i>Staphylococcus capitis</i>	-	-	-	7	11	-	5	10	12	15	22
<i>Staphylococcus epidermidis</i>	-	-	-	9	10	-	-	5	10	12	16
<i>Staphylococcus aureus</i>	2	4	10	12	20	2	5	11	15	23	22
<i>Proteus sp.</i>	-	-	-	10	18	-	-	5	11	16	18
<i>Proteus mirabilis</i>	-	-	-	7	11	-	-	5	6	13	15
<i>Proteus vulgaris</i>	-	3	4	10	18	-	-	10	17	21	22
<i>Klebsiella sp.</i>	2	5	8	11	17	2	5	11	16	18	24
<i>Klebsiella pneumoniae</i>	-	-	-	4	10	-	2	6	8	16	22
<i>Klebsiella oxytoca</i>	-	-	-	7	8	-	-	5	5	9	18
<i>Morganella morganii</i>	2	7	10	14	16	5	10	16	18	22	16
<i>Micrococcus</i>	R	R	R	R	R	-	-	-	-	5	22
<i>Citrobacter freundii</i>	2	4	7	10	14	-	2	5	12	15	24
<i>Pseudomonas sp.</i>	-	-	10	15	20	-	-	9	16	21	18
<i>Pseudomonas aeruginosa</i>	-	-	10	15	21	-	6	10	18	22	14
<i>Pseudomonas luteola</i>	-	-	-	3	5	-	-	2	4	7	16

The antibacterial activity of Watery Parsley extract of all bacteria revealed that most isolate showed inhibition zone to (2-21) mm. There no effects of these extract against *Micrococcus*, *P. aeruginosa* and *Klebsiella oxytoca*. The result of antibacterial activity of Alcohol Parsley extract of all bacteria revealed that the inhibition zones between (2-22) mm. There low effect of this extract against *Micrococcus* in (75-100) concentration only. Table (4).

Table (4) Antibacterial activity of Parsleys at different concentration against different bacteria.

Bacteria isolate	Watery Parsleys extract Inhibition zone in (mm)					Alcoholic Parsleys extract Inhibition zone in (mm)					Antibiotic Impinim (Control)
	Concentration of extract										
	12.5	25	50	75	100	12.5	25	50	75	100	
<i>E. coli</i>	-	4	10	11	12	-	6	11	12	14	18
<i>Staphylococcus sp.</i>	-	-	8	10	10	-	-	8	10	13	18
<i>Staphylococcus albus</i>	2	6	9	10	11	-	7	9	11	13	20
<i>Staphylococcus capitis</i>	-	6	8	10	10	2	7	11	13	14	22
<i>Staphylococcus epidermidis</i>	-	4	6	8	8	-	3	8	10	10	16
<i>Staphylococcus aureus</i>	3	7	9	11	12	3	7	8	12	13	22
<i>Proteus sp.</i>	2	5	12	14	21	6	10	12	17	22	18
<i>Proteus mirabilis</i>	-	4	10	13	16	6	8	16	18	20	15
<i>Proteus vulgaris</i>	2	6	10	12	18	6	10	14	15	22	22
<i>Klebsiella sp.</i>	-	-	5	10	15	-	4	10	15	17	24
<i>Klebsiella pneumoniae</i>	-	-	5	10	11	-	-	-	12	13	22
<i>Klebsiella oxytoca</i>	R	R	R	R	4	-	-	-	5	8	18
<i>Morganella morganii</i>	-	-	2	6	6	-	-	5	7	8	16
<i>Micrococcus</i>	R	R	R	R	6	R	R	R	6	8	22

<i>Citrobacter freundii</i>	R	R	7	10	10	-	4	10	13	14	24
<i>Pseudomonas sp.</i>	-	-	-	5	6	-	-	-	5	7	18
<i>Pseudomonas aeruginosa</i>	-	-	5	8	8	-	2	6	10	11	14
<i>Pseudomonas luteola</i>	R	R	R	R	R	R	R	R	4	4	16

Discussion:-

In our study 120 (66.66%) isolate include *E. coli* was most predominant uropathogen with(46.66%), followed by *Staphylococcus aureus* (15.8%), *Staphylococcus sp.* and *Proteus spp.* (5%) *Klebsiella sp* and *Pseudomonas aeruginosa* (4.16%) each of them. *Klebsiella pneumoniae* and *Pseudomonas sp.* (3.33%), *Proteus mirabilis* (2.5%) *Staphylococcus albus*, *Morganella morganii* and *Micrococcus* (1.66%) each of them. *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Klesiella oxytoca*, *Citrobacter freundii* and *Pseudomonas luteola* (0.83%). These result agree with Shaaban *et al.*, 2012 who found that *E. coli* 43% followed by *Klebsiella pneumoniae*14.1%, *Pseudomonas aeruginosa* and *Proteus mirabilis* 9.4 %, *Staphylococcus aureus* 7.8%, *Morganella morganii* 6.2%. In sectional study by university of Florida USA of a group of patient, 81 patients met the inclusion criteria of this study of these 81 patients 89% had UTIE. *coli* (McCloughlin and Joseph, 2003 and Abd, 2012).

In this study, the prevalence of UTI was in female more than in male (66.6% female and 33.33% male) in another study showed females 63.6% and 36.4% male, it was seen that significantly higher incidence rate of girls than for boys. Another study also showed that urinary tract infection are more common in girls (Shaaban *et al.*, 2012).

Antibiotic provide the main basis for the therapy of microbial infection , since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious disease , But worldwide emergence of resistant bacteria has become a major therapeutic problem at the recent time , In addition multidrug resistant strains are also increasingly being isolated from community acquired infections (Fuad *et al.*, 2012)

All isolate was multi drug resistant against antibiotic. All bacteria of *E. coli* , resistance to all antibiotic and sensitive to Meropenem , Nitrofurantoin , Gentamicin , Aztreonam , and Co-trimoxazole , *Morganella morganii* resistance to all antibiotic and sensitive to Ampicillin, Aztreonam, Azithromycin, Ciprofloxacin, Gentamicin, Impinim , Meropenem and Nitrofurantoin. *Micrococcus* resistance to all antibiotic and sensitive to Amoxicillin/clavulanic acid , Ampicillin, Aztreonam ,Cephathiane, Cefixime .Ciprofloxacin , Impinim, Nalidixic acid , Nitrofurantoin *Citrobacter frenudii* resistance to all antibiotic and sensitive to AT, Aztreonam ,Azithromycin, Ceftazidime , Ciprofloxacin , Impinim, Meropenem Nalidixic acid , Nitrofurantoin. All bacteria *Staphylococcus sp.* were resistance to Amoxicillin/clavulanic acid , Ampicillin , Aztreonam , Meropenem , Azithromycin , Ceftazidime , Ceftridacin , Cyclodextrin , AT , Ciprofloxacin , Co-trimoxazole, Cefixime , Cephathiane and sensitive to Amikacin , Impinim, Gentamicin , Nalidixic acid, Nitrofurantoin. *Proteus sp.* showed resistance to all antibiotic only Aztreonam, Ciprofloxacin, Meropenem. *Pseudomonas sp.* showed sensitive only to Amikacin AT, Ciprofloxacin, Gentamicin Meropenem and Impinim. *Klebsiell sp.* were resistance against all antibiotic while sensitive against Aztreonam ,Gentamicin , Meropenem, Nalidixic acid , Nitrofurantoin and Impinim. This is similar to the study of (Tyagiet *al.*, 2011) in which gram negative bacteria isolate of UTI were multi drug resistant to Ampicillin, Amoxicillin, Ceftizoxime, Cefepime, Tetracyclin. Many studies have shown that active efflux can be a mechanism of resistance for almost all antibiotics (Adwanet *al.*, 2009). In another study showed all isolates were resistant to one or two of cephalosporin, penicillin and β - lactam groups. Resistance of bacteria means that these bacteria have antibiotic resistance genes. Increasing of infections based on antibiotic resistant microorganisms has to be using new and natural antimicrobials. Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development. (Akrayi and Abdulrahman 2013)

The our result of antibacterial activity of Watery Celery extract of all bacteria revealed that the inhibition zone (2-20) mm, while there were no effect up to *Micrococcus*. Antibacterial activity of Alcohol Celery extract of all bacteria produced inhibition zones between (2-23) mm.

The antibacterial activity of Watery Parsley extract of all bacteria revealed that most isolate showed inhibition zone to (2-21) mm. There no effects of these extract against *Micrococcus*, *P. aeruginosa* and *Klesiella oxytoca*. The result of antibacterial activity of Alcohol Parsley extract of all bacteria revealed that the inhibition zones between (2-22) mm. There low effect of this extract against *Micrococcus* in (75-100) concentration only. Results of Shaaban *et al.*, 2012 also indicated the effect of parsley and celery , their stronger effects were against gram positive cocci follower by gram negative bacilli while their effect on *E. coli* was much less . Parsley and celery followed dill in

their general effect. They affected (41% and 34%) of the local uropathogens respectively. Our results agreement also with Kareemi, 2012 who showed that most bacterial isolates have inhibition zone to Parsley juice with different diameters and the inhibitory effect lasted up to 1:1 dilution, while there were no effect by each of 1:5, 1:10, 1:15 and 1:20. *P.aeruginosa* was the only isolates that showed resistant to all different Parsley juice dilutions. In another study of Seyyedejad *et al.*, 2008 showed that ethanolic extracts from the parsley inhibited the growth of various species of gram positive and gram negative bacteria the parsley ethanolic extract inhibition the growth of 8 out of 11 bacteria species. Therefore Medicinal plants could be one approach because most of them are safe with little side effects if any cost less and affect a wide range of antibiotic resistant microorganisms Medicinal plants have been used in traditional medicine for the treatment of urinary tract disease. At the present time, the interest in the folk medicine is increasing because many patients believed that such products are effective and less harmful. (Naema *et al.*, 2010)

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