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ANTIBACTERIAL ACTIVITY OF *ZEA MAYS L* AND *URTICA DIOICA L* EXTRACT ON THE ISOLATED BACTERIA FROM CHILDREN UTI IN ERBIL CITY

***Sawsan Mohammed Abdullah Surjee, **Luma Abdal Hady Zwain**

**Biology Department, College of Education/Scientific Departments, University of Salahaddin, Erbil, Iraq.*
***Biology Department, College of Education for Pure Science Ibn-Al-Haitham, University of Baghdad, Iraq.*

ABSTRACT

The antibacterial effect of ethanolic and aqueous extracts of Zea mays L and urtica dioica L, was investigated on bacteria were obtaind from human Urinary Tract Infection (UTI) from Hawlery Ferkary Hospital in Erbil City- Iraq, by using the well diffusion technique .A 120 isolates bacteria isolated from 180 UTA patient, isolates were identified as E.coli, Staph. spp, Staph. albus, Staph. capitis, Staph epidermis, Staph. aureus, Pseudo. spp., Pesudo. lutela, Pseudo. aeruginosa, Proteus. spp., P. mirabilis, Morganella, Morganii, Klebsiella spp., K. Pneumonia, K. oxytoca, Micrococcus and Citrobacter frenudii following different morphological, physiological and biochemical test. Antibiotic sensitivity of 17 commerical antibiotic discs AK,AMC, AMP, AT, ATM, AZM, CAZ, CD, CEP, CFM , CIP, COT, CRO, GM, IPM, MEM and NA was screened by disc diffusion assay , It was observed that the extracts of both plant had an inhibitory effect on the bacteria under study, except Pesudo. lutela to extracts of Zea mays L. and Pesudo. lutela, K. Pneumonia and K. oxytoca to extracts of Urtica dioica L. The inhibitory concentration of of Zea mays L. extracts was 75% and 100% and Urtica dioica L. extracts was 50%, 75% and 100%. The antibacterial activity of extracts of U. dioica showed the best action as inhibitor against test bacteria than the extracts of zea mays. The result showed there is antibacterial activity of alcohol extract of U. dioica higher sensitivity against a number of bacteria compared with standard antibiotics

INTRODUCTION

More than 1500 herbal preparations are sold as dietary supplements or ethnic traditional medicines (1). (1)There are several reports on antimicrobial activity of crude extracts prepared from plants that inhibit various bacterial pathogens, because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, phenolic compounds, which are part of essential oils, Tannin, terpenoids, alkaloids, and flavonoids(2). The antimicrobial activity of different plant species in various geographical regions in search for new antibiotics (3). In recent years, human pathogens have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Undesirable side effects of certain antibiotics and the emergence of previously uncommon infections led the scientists to look for new antimicrobial substance from various sources, especially from medicinal plants. The screening of plant

extracts and products presents potential sources of new antimicrobial agents (4,5). Urinary tract infection (UTI) is one of the most common causes of hospitalization and referral to outpatient settings in children. It is estimated that at least 3% of girls and 1% of boys experience one episode of UTI before the 11th years of age (6). About 30-50% of these patients will have another episode within three months to two years (6). Early treatment of UTI with an effective antibiotic is essential for prevention from long-term consequences. Delay in treatment increases the risk of scar formation in kidneys (7). Common nettle (*Urtica dioica* L.), a herbaceous perennial flowering plant, is a member of the *Urticaceae* family. Traditional herbal medicine in the Balkan countries uses stinging nettle leaves in the form of an herbal infusion as a remedy for the treatment of diarrhea, vaginal discharge, internal/external bleeding (8). Being rich in chlorophyll, nettle leaves are used for the treatment of anemia as well as general well-being, and more recently as natural food colorant (8). *Zea mays* L. is fine soft thread 10-20 cm long, commonly cultivated in warm climates. It is medicinally used as a mild stimulant, diuretic and demulcent, useful in acute and chronic cystitis and in the bladder irritation of uric acid and phosphatic gravel; has also been employed in Gonorrhoea [9]. In Chinese medicine, *Zea mays* L. is used for oedema of various origin and for hepato-biliary disease [10]. The medicinal properties of *Zea mays* L. supported by several authors as it exhibited antioxidant activity (11)anti-diabetic activity [12], antibiotic activity towards corn earworm [13, resistance to insect attacks [14] and antitumor activity [15]. Phytochemical studies on *Zea mays* L. revealed that it contained a number of flavonoids, chlorogenic acid, p-coumaric, ferulic acid, saponins, phytosterols, volatile oil, fixed oil, resin, sugars, allantoin, tannin and minerals [16,17].

MATERIALS AND METHODS

Bacteria tested

The bacteria under study (*E.coli, Staph .spp, Staph. albus, Staph .capitis, Staph epidermis, Staph. aureus, Pseudo.* spp., *Pesudo lutela, Pseudo.aeruginosa, Proteus.* spp., *P. mirabilis, Morganella morganii, Klebsiella* spp., *K. Pneumonia, K. oxytoca, Micrococcus* and *Citrobacter frenudii*) were obtained from human Urinary Tract Infection (UTI) from Hawlery Ferkary Hospital in Erbil City–Iraq. The isolates were inoculated on agar to obtain a single colony, which were subcultured on the same medium to check for the purity of the isolated bacteria. Purified iso- lates were identified using morphological, cultural, and some bio- chemical tests, as a more accurate method for identification (2).

Plant Extraction

Collection and preparation of plant samples both plants Nettle leaves (*Urtica dioical* L.) and Corn silk (*Zea mays* L.) with tap water using soap powder, and then were washed with distilled water. They were then air dried, powdered, and stored in polyethylene bags in refrigerator at 4°C for further processes (2).

Extract preparation

The ethanolic and aqueous extracts of both plants were pre-pared by maceration method according to the procedure discussed in Reference 11 with slight modification. 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 further 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 (2).

Well diffusion technique

Screening of antibacterial activity was performed by well dif-fusion technique (13). The Nutrient agar (NA) plates were seeded with 0.1 ml of the inoculums of each tested organism. The inoculums were spread evenly over the plates with a loop. A standard cork borer of 8-mm diameter was used to cut uniform wells on the surface of the NA, and 100 µl of each concentration of plant extracts or juices was introduced in the well. The plates were incubated for 24 hours at 37°C, and the zones of inhibition was measured to the nearest millimeter (mm). (2)

RESULT

One hundred and eighty (180) Swabs were collected from patients (>1-19) years old infected with obtained from human Urinary Tract Infection (UTI) from Hawlery Ferkary Hospital in Erbil City- Iraq120 isolates of gram positive bacteria were obtained from these samples and were diagnosed , of which *E. coli* comprised the highest percentage 56 (46.66%), *Staphylococcus aureus* was 19 (15.8%), 0.83-5% other type of bacteria as *E. coli*, *Staph .spp*, *Staph. albus*, *Staph. capitis*, *Staph. epidermis*, *Staph. aureus*, *Pseudo.* spp., *Pesudo. lutela*, *Pseudo. aeruginosa*, *Proteus.* spp., *P. mirabilis*, *Morganella*, *Morganii*, *Klebsiella.* spp., *K. Pneumonia*, *K. oxytoca*, *Micrococcus* and *Citrobacter frenudii* (Fig 1). 23 (19.6) collected from patients (>1) years old, 18 (7.5-15%) from (1-5) years old, 2-8 (1.66-6.66%) from (6-19). (Fig2). 80 (6.66%) female and 40 (33.3%) male (Table 1).

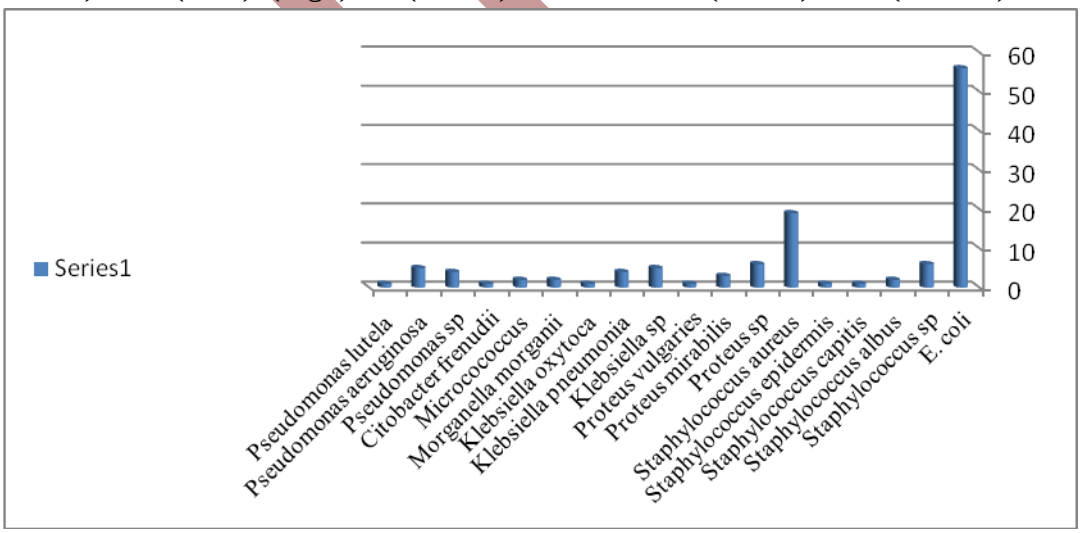


Fig 1: Number of Bacteria isolates from UTI

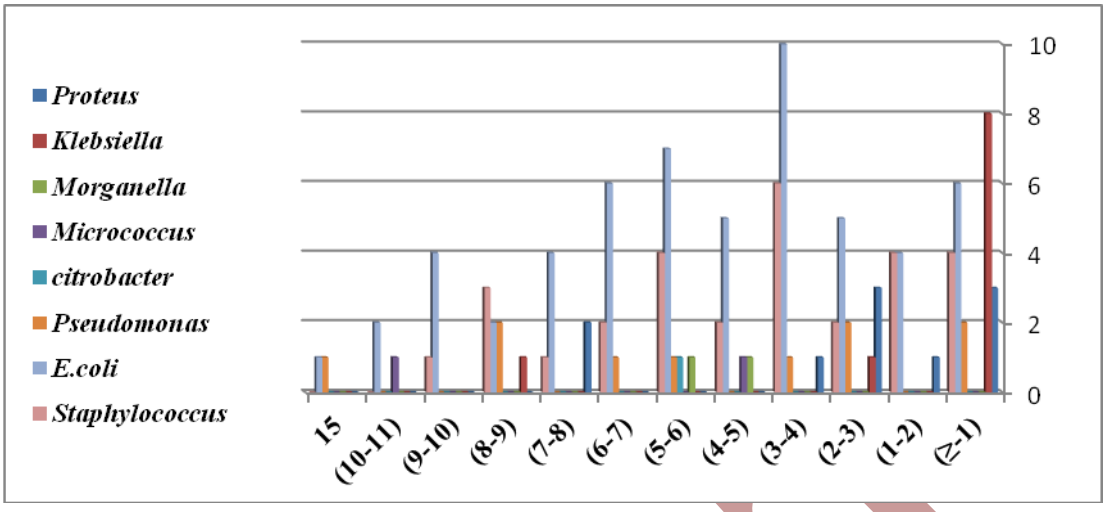
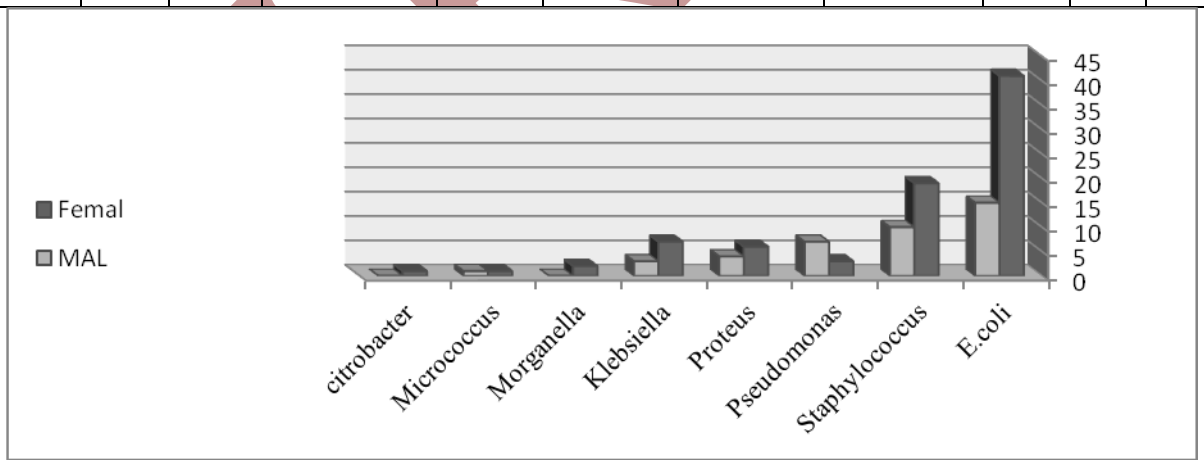


Fig 2: Percentage bacteria isolate of different age

Table 1: Percentage bacteria isolate of different mal and femal

	E.coli	*Staph.*	*Pseudomonas.*	*Proteus*	*Klebsiella.*	*Morganella*	*Micrococcus*	*Citro.*	**total**	**%**
female	41	19	3	6	7	2	1	1	80	66.6
male	15	10	7	4	3	0	1	0	40	33.3
total	56	29	10	10	10	2	2	1	120	99.9



In the present investigation (Table 2) the inhibitory effect of crude aqueous and ethanolic extracts of sik of zea mays against bacterial isolated from UTI,results showed that aqueous extract possess strong antibacterial activity against *Staph. capitis*, *Staph.aureus* and *Speudo. aeruginosa* while moderate against *Staph. albus*, *Morganella*, *Morganii*, *E.coli*, *Staph. epidermis*, *Micrococcus*, *K. Pneumonia* and *Citrobacter frenudii* and weak against *Proteus. Spp* , *P. vulgaries* , *Klebsiella.* spp., *P. mirabilis*, *K. oxytoca* , *Staph.* spp. and *Pseudo.* spp. Ethanolic extracts was strong antibacterial activity against *Staph.* spp, *Staph.*

capitis, Staph. albus, *Morganella, Morganii* and *Staph. aureus* while moderate against *Pseudo.* spp., *Citrobacter frenudii*, *Proteus.* spp., *E.coli*, *P. mirabilis* , *P. Vulgaries*, and weak against *K. oxytoca* at concentration of 100% showed a zone of inhibition in the concentration till reach to height of 16 mm to aqueous extracts and 20 mm to ethanolic extracts .All bacterial species included in the test showed effected toward aqueous and ethanolic extracts of sik of zea mays under study except *Pesudo. lutela*. It was also observed that the ethanolic extracts acted as better antibacterial agents than the aqueous extracts, ethanolic and aqueous extracts were less or no antibacterial activity at concentration of 25 and 12.5 . The inhibitory effect of crude aqueous of *Urtica urins* possess strong antibacterial activity against *Citrobacter frenudii, Proteus.* spp., *P. vulgaries, Staph. epidermis, Staph.* spp., *Staph. aureus, Staph.* spp., *Speudo. aeruginosa* and *P. vulgaries* and weak against *Micrococcus, E. coli, Morganella, Morganii* and *Klebsiella.* spp., while no effect moderate against any bacteria. Ethanolic extracts was strong antibacterial activity against *Proteus.* spp., *P. mirabilis, E. coli, Citrobacter frenudii, Pseudo.* spp., *Staph. albus, Staph. epidermis, Staph.* spp., *Staph. aureus, Speudo. aeruginosa* and *P. vulgaries* while moderate against, *Morganella Morganii, Micrococcus* and *Klebsiella.* spp. All bacterial species included in the test showed effected toward aqueous and ethanolic extracts of *Urtica urins* under study except *K. oxytoca* and *K. Pneumonia*. It was also observed that the ethanolic extracts acted as better antibacterial agents than the aqueous extracts, aqueous extracts were less or no antibacterial activity at concentration of 50, 25 and 12.5 %, at concentration of 100% showed a zone of inhibition in the concentration till reach to height of 20 mm to aqueous extracts and 38 mm to ethanolic extracts . The antibacterial activity of *Urtica dioica* L. than that of sik of *Zea mays*. It is noted extracts of *Urtica dioica* L., especially the ethanolic extracts, had maximum antibacterial activity. The results (as in Tables 3) showed that almost bacteria under study were resistant to more than six antibiotics , where this property refers that these G - ve bacteria are multiple drug resistance (MDR) .In case of comparing the results showed in (Table 2, 3) with

Table 2: Showed the inhibition zone produced by using the extracts of (*Zea mays* L.).

	Concentration of aqueous %					Concentration of alcoholic %					IPM
	100	75	50	25	12.5	100	75	50	25	12.5	
E.coli	5	5	2	-	-	30	25	22	7	4	S
Staph .spp	12	10	8	-	-	20	18	12	6	-	S
Staph. albus	11	11	7	4	-	23	20	12	7	-	S
Staph epidermis	14	13	8	4	-	21	19	10	5	2	S
Staph. aureus	13	12	9	-	-	19	14	14	8	4	S
Pseudo. Spp	3	-	-	-	-	25	20	20	5	-	S
Pesudo lutela	-	-	-	-	-	8	5	-	-	-	S
Pseudo.aeruginosa	12	5	-	-	-	19	19	12	5	-	S
Proteus. Spp	20	19	12	8	4	38	35	30	18	8	S
P. mirabilis	20	17	11	6	3	32	27	24	14	5	S
P. Vulgaries	12	10	7	-	-	18	15	10	5	-	S

Morganella.Morganii	**4**	**-**	**-**	**-**	**-**	**10**	**7**	**4**	**-**	**-**	**S**
Micrococcus	**8**	**6**	**4**	**-**	**-**	**10**	**7**	**4**	**-**	**-**	**S**
Klebsiella .Spp	**2**	**2**	**-**	**-**	**-**	**8**	**2**	**-**	**-**	**-**	**S**
K. Pneumonia	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**S**
K. oxytoca	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**S**
Citrobacter frenudii	**22**	**20**	**12**	**7**	**2**	**26**	**22**	**15**	**6**	**3**	**S**

-No inhibition zone appeared

Table 3: Showed the inhibition zone produced by using the extracts of (*Urtica dioical* L.)

	Concentration of aqueous extract %					**Concentration of alcoholic extract %**					**IPM**
	100	**75**	**50**	**25**	**12.5**	**100**	**75**	**50**	**25**	**12.5**	
E.coli	**8**	**7**	**6**	**2**	**-**	**9**	**7**	**4**	**2**	**-**	**S**
Staph .spp	**3**	**3**	**-**	**-**	**-**	**20**	**12**	**8**	**-**	**-**	**S**
Staph. albus	**10**	**7**	**4**	**-**	**-**	**13**	**10**	**9**	**6**	**-**	**S**
Staph .capitis	**16**	**11**	**7**	**5**	**-**	**17**	**15**	**10**	**4**	**-**	**S**
Staph epidermis	**8**	**6**	**-**	**-**	**-**	**8**	**7**	**4**	**-**	**-**	**S**
Staph. aureus	**12**	**10**	**4**	**-**	**-**	**12**	**11**	**8**	**5**	**2**	**S**
Pseudo. Spp	**2**	**5**	**-**	**-**	**-**	**10**	**6**	**5**	**-**	**-**	**S**
Pesudo lutela	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**S**
Pseudo.aeruginosa	**12**	**8**	**4**	**-**	**-**	**13**	**11**	**7**	**-**	**-**	**S**
Proteus. Spp	**7**	**5**	**-**	**-**	**-**	**10**	**9**	**5**	**-**	**-**	**S**
P. mirabilis	**5**	**3**	**2**	**-**	**-**	**9**	**5**	**4**	**-**	**-**	**S**
P. Vulgaries	**7**	**6**	**5**	**-**	**-**	**9**	**4**	**-**	**-**	**-**	**S**
Morganella.Morganii	**10**	**9**	**4**	**-**	**-**	**13**	**12**	**8**	**5**	**-**	**S**
Micrococcus	**8**	**8**	**7**	**-**	**-**	**9**	**7**	**4**	**2**	**-**	**S**
Klebsiella .Spp	**6**	**-**	**-**	**-**	**-**	**9**	**7**	**2**	**-**	**-**	**S**
K. Pneumonia	**8**	**6**	**-**	**-**	**-**	**8**	**7**	**4**	**-**	**-**	**S**
K. oxytoca	**4**	**-**	**-**	**-**	**-**	**7**	**5**	**-**	**-**	**-**	**S**
Citrobacter frenudii	**8**	**6**	**-**	**-**	**-**	**10**	**8**	**5**	**2**	**-**	**S**

- No inhibition zone appeare

Tables (4) *E.coli* was resistant to three antibiotics (AMP, CD and NA) and susceptible to five antibiotics (AK, ATM, COT, GM and MEM). *Citrobacter frenudii* was resistant to eight antibiotics (AK, AMC, AMP, CD, CEP, CIP, COT, and GM) and susceptible to five antibiotics (AT, ATM, AZM, CAZ and CFM). *Morganella, Morganii* was resistant to three antibiotics (CAZ , CDA and NA) and susceptible to seven antibiotics(AMP, ATM, AZM, CIP, GM, IPM and MEM). *Micrococcus* was resistant to seven antibiotics (AT, AZM, CAZ, CD, COT, COR and GM) and susceptible to eight antibiotics (AMC, AMP, ATM, CEP, CFM , CIP, IPM and NA). *Staph.* spp. was resistant to three antibiotics (COT, CRO and GM) and susceptible to (AK, AT, CD, CEP, IPM and MEM). *Staph. albus* was resistant to six antibiotics (AMP, AMP, ATM, AZM, CAZ, CFM and CIP) and susceptible to (AK, AT,

CEP, COT, CRO, GM, IPM and MEM). *Staph. capitis* was resistant to eight antibiotics (AK, AMC, AMP, AZM, CAZ, CD, CEP and CRO) and susceptible to (AT, ATM, CFM, CIP,COT, GM, IPM and MEM). *Staph. epidermis* was resistant to 11antibiotics (AMC, AMP, AT, ATM, AZM, CAZ, CEP, CFM, COT, CRO and MEM) and susceptible to (AK, CD, GM, and IPM). *Staph.aureus* was resistant to five antibiotics (AT, ATM, CAZ CFM and CIP) and susceptible to (AK, AMC, CD ,CEP, COT, IPM and MEM). *Proteus.* spp. was resistant to five antibiotics (AZM , CFM, CRO, GM and IPM) and susceptible to (AK, ATM, CIP and MEM) *P. mirabilis* was resistant to five antibiotics (AMP, AT, AZM, CD and CEP). and susceptible to (ATM, CAZ, CFM, CIP, GM, IPM and MEM). *P. vulgaries* was resistant to nine antibiotics (AMP, ATM, CAZ, CD, CEP, CFM, CRO, GM and NA) and susceptible to (AK, AMC, AT, AZM, CIP, COT, IPM and MEM). *Pseudo.* spp. was resistant to six antibiotics (AMC, AMP, ATM, CD, COT and NA) and susceptible to (AK, AT, CIP, GM, IPM and MEM). *Peudo. aeruginosa* was resistant to six antibiotics (AMC, AMP, CD, CEP, CFM and GM) and susceptible to (AT, ATM, AZM, CIP, COT, GM, IPM and MEM). *Pesudo. lutela* was resistant to eight antibiotics (AMC, AMP, ATM, AZM, CAZ, CD, CEP, CFM) and susceptible to (AK, AT , CIP, GM, IPM, MEM and NA). *Klebsiella.* spp. was resistant to seven antibiotics (AMC,AMP,AT, CAZ, CD , CEP and CRO). and susceptible to (AK, ATM, CIP, IPM and MEM). *K. Pneumonia* was resistant to three antibiotics (AMP, AT and CD) and susceptible to (ATM, CIP, GM, IPM, MEM and NA). *K. oxytoca* was resistant to ten antibiotics (AK, AMC, AMP, AT, CAZ, CD , CFM, COT and CRO) and susceptible to (ATM, AZM, GM, IPM, MEM and NA). Table 2 and 3 the antibacterial activity of extracts of *U. dioica* showed the best action as inhibitor against test bacteria than the extracts of zea mays .This is observed in other studies as well (18) in which the alcohol extract of *U. dioica* inhibit the growth of gram positive and negative bacteria and confirm that alcohol extract has more activity than the water extracts , this may be attributed to the difference in the activity of the active compounds when extracted with different solvents (19) but (20) observed the *U.dioica* extract exhibited best antibacterial activity against the *B. subtillis* and *E.coli* with the lowest inhibitory activity against *Peudo. aeruginosa*, water extract had no effect on the growth of *Peudo. Aeruginosa*. The total phenolic content in ethanol extract of *U. dioica* is high and flavonoid and flavonols is low (20) .The result showed there is antibacterial activity of alcohol extract of *U. dioica* higher sensitivity against a number of bacteria compared with standard antibiotics, the extract higher sensitivity against of *Staph.* spp. than (COT, CRO, GM). Of *Staph. albus* than (AMP, AMP, ATM, AZM, CAZ, CFM and CIP). Of *Staph. epidermis* than (AMC, AMP, AT, ATM, AZM, CAZ, CEP, CFM, COT,CRO and MEM) .Of *Pseudo.* spp. than (AMC, AMP, ATM, CD, COT and NA). Of *Proteus.* spp. than (AZM , CFM,CRO,GM and IPM) . Of *P. mirabilis* than (AMP, AT, AZM,CD and CEP). Of *Citrobacter frenudii* than (AK, AMC, AMP, CD, CEP, CIP, COT, and GM). It is noted from the present result that the extracts of *Zea mays* had minimum antibacterial activity, which is don't identical with results obtaind from other researchers (21). Extract and flavonids of *Zea mays* showed higher sensitivity against a number of bacteria than gentamysin (22) However, negative results do not indicate that the bioactive constituents are absent or that the plant is inactive. Active compounds may be present in insufficient quantities in the crude

extracts; therefore, the dose levels employed would not be sufficient enough to exhibit the inhibitory activity. The lack of inhibitory activity can thus only be proven by using large doses. Alternatively, even if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects on the positive effects of the bioactive agents, thus zeroing the antibacterial activity of the principle. It is also possible that the extracts may be active against other bacterial species that were not tested (22).

Table 4 : Resistance of bacteria under study to antibiotic

Isolate bacteria	Ak	AMC	AMP	AT	ATM	AZM	CAZ	CD	CEP	CFM	CIP	COT	CE
E. coli	33.9	66	93	40	13	43.7	52	98	59	63	60	21	6
Citobacter frenudii	100	100	100	0	0	0	0	100	100	0	100	100	0
Micrococcus	50	0	0	100	0	100	100	100	0	0	0	100	10
Morganella morganii	50	50	0	50	0	0	100	100	50	50	0	50	5
Staphylococcus sp	20	66.6	75	0	50	66.6	60	33.3	17	40	80	100	10
Staphylococcus albus	0	50	100	0	100	100	100	50	0	100	100	0	0
Staphylococcus capitis	100	100	100	0	0	100	100	100	100	0	0	0	10
Staphylococcus epidermis	0	100	100	100	100	100	100	0	100	100	30	100	10
Staphylococcus aureus	33.3	33.3	64	100	87	79	95	22.2	25	85	85	10	5
Proteus sp	0	60	50	50	0	80	60	75	75	100	0	70	10
Proteus mirabilis	70	70	100	100	0	100	33	100	100	0	0	70	5
Proteus vulgaries	0	0	100	0	100	0	100	100	100	100	0	0	10
Pseudomonas sp	0	100	100	0	100	50	75	100	50	75	0	100	7
Pseudomonas aeruginosa	50	100	100	0	0	25	50	100	100	100	0	33	10
Pseudomonas lutela	0	100	100	0	100	100	100	100	100	100	0	100	10
Klebsiella sp	25	100	100	100	0	60	100	100	100	80	0	75	10
Klebsiella pneumonia	50	75	100	100	0	70	70	100	70	70	0	50	7
Klebsiella oxytoca	100	100	100	100	0	0	100	100	100	100	50	100	10

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: Ceftridacim , GM: Gentanmicin, IPM: Impinem, MEM: Meropenem, NA: Nalidixic acid , NIT: Nitrofuration.

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