RESULTS

1- Collection of urine samples from males and females.

The present study is conducted on 138 urine samples were taken from patients who were examinated for UTIs in Zagazig University hospitals (inpatients) or attending Zagazig University clinics (outpatients), Urology Department. These samples have been collected from different ages of males and females ranged 35-75 years of males and 20-60 years of females. Forty seven patients of these cases were females and fifty three were males. These samples were collected from April 2007 to August 2007.

The diagnosis of UTIs as positive urine sample was based on the presence of $\geq 10^5$ CFU of microorganisms per ml in urine culture. Also, presence of more than 5 pus cells per high power field in an unspun urine in male, and more than 10 pus cells in a female, and is defined as pyuria. High number of pus cells in urine, or pyuria, usually indicates infection.

The obtained results in table (1) illustrated that 100 urine samples out of 138 urine samples were positive for urinary tract infection, and 38 samples were negative (urinary tract infection not present). Total bacterial counts ranged from 10×10^4 to 16×10^4 cfu/ml of urine and these samples collected from 63 outpatient and 37 inpatient and pus cells ranged from 40 to > 100 hpf/ml of urine samples.

As illustrated in table (2), the highest number of urine male's samples was collected from 56-60 years age which collected 23 samples and the infected urine positive samples were 16 with 69.56% positive percentages and the maximum infected positive percentage urine samples were obtained from male age from 41-45 and age 71-75 with 83.33%. And the highest number of urine female's samples was collected from 20-25 years age which collected 16 samples and the infected urine positive samples were 12 with 75.00% positive percentages and the maximum infected positive percentage urine samples were obtained from female age 51-55 with 85.71%.

The statistical analysis for these results were performed according to age, sex, personal state, pregnant and non-pregnant women, as illustrated in table (3), where the positive collected samples of males 53 more than the females samples 47 and the infection of married (male & female) was the highest which recorded 92 positive samples if compared with single which recorded 8 positive samples.

Table (1): Positive urine samples collected from different ages of males and females patients.

Urine samples No.	Age	Gender	Personal state	Pregnant female	Outpatients & inpatients	Pus Cells No. per (hpf)	Total bacterial Count (cfu/ml)	Specific risk factors
1	63	M	Married		Outpatient	> 100	16×10^4	Diabetes
2	20	F	Single	No	Outpatient	> 100	13×10^4	K.S
3	39	M	Married		Outpatient	80	12×10^4	
4	25	F	Married	Yes	Outpatient	70	12×10^4	
5	40	M	Married		Outpatient	40	10×10^4	
6	21	F	Married	Yes	Outpatient	40	10×10^4	
7	21	F	Single	No	Outpatient	70	11×0^4	
8	59	M	Married		Outpatient	70	12×10^4	
9	25	F	Married	Yes	Outpatient	50	12×10^4	
10	25	F	Married	No	Outpatient	80	11×10^4	
11	21	F	Single	No	Outpatient	45	10×10^4	
12	20	F	Single	No	Outpatient	60	11×10^4	
13	21	F	Single	No	Outpatient	90	11×10^4	
14	23	F	Married	No	Outpatient	70	11×10^4	
15	20	F	Single	No	Outpatient	60	10×10^4	
16	20	F	Single	No	Outpatient	60	11×10^4	
17	27	F	Married	Yes	Outpatient	80	12×10^4	
18	75	M	Married		Outpatient	> 100	12×10^4	Diabetes
19	29	F	Married	Yes	Outpatient	40	11×10^4	
20	57	M	Married		Outpatient	40	10×10^4	
21	28	F	Married	No	Outpatient	60	10×10^4	
22	27	F	Married	No	Outpatient	75	10×10^4	
23	58	M	Married		Outpatient	> 100	12×10^4	
24	60	M	Married		Outpatient	45	10×10^4	
25	28	F	Married	Yes	Outpatient	60	11×10^4	
26	58	M	Married		Outpatient	> 100	12×10^4	Diabetes
27	26	F	Married	Yes	Outpatient	60	11×10^4	
28	59	M	Married		Outpatient	> 100	12×10^4	K.S
29	29	F	Married	No	Outpatient	45	10×10^4	
30	30	F	Married	Yes	Outpatient	40	13×10^4	

31	26	F	Single	No	Outpatient	55	10×10^4	
32	60	M	Married		Outpatient	> 100	11×10^4	
33	27	F	Married	No	Outpatient	40	10×10^4	
34	65	M	Married		Outpatient	> 100	11×10^4	K.S
35	71	M	Married		Outpatient	> 100	11×10^4	K.S
36	31	F	Married	No	Outpatient	> 100	16×10^4	K.S
37	45	M	Married		Outpatient	> 100	11×10^4	K.S
38	42	M	Married		Outpatient	> 100	11×10^4	Diabetes
39	65	M	Married		Outpatient	40	10×10^4	
40	32	F	Married	Yes	Outpatient	60	13×10^4	
41	50	M	Married		Outpatient	60	10×10^4	
42	49	M	Married		Outpatient	> 100	11×10^4	K.S
43	39	F	Married	No	Outpatient	45	11×10^4	
44	55	F	Married	No	Outpatient	> 100	15×10^4	K.S
45	42	F	Married	No	Outpatient	40	10×10^4	
46	44	F	Married	No	Outpatient	40	11×10^4	
47	50	F	Married	No	Outpatient	> 100	15×10^4	K.S
48	58	M	Married		Outpatient	> 100	13×10^4	K.S
49	46	F	Married	No	Outpatient	60	11×10^4	
50	48	F	Married	No	Outpatient	55	10×10^4	
51	57	F	Married	No	Outpatient	> 100	14×10^4	Diabetes
52	42	F	Married	No	Outpatient	40	10×10^4	
53	59	F	Married	No	Outpatient	75	12×10^4	
54	58	F	Married	No	Outpatient	45	10×10^4	
55	55	F	Married	No	Outpatient	80	12×10^4	
56	50	M	Married		Outpatient	60	12×10^4	
57	58	M	Married		Outpatient	> 100	14×10^4	Diabetes
58	55	F	Married	No	Outpatient	> 100	12×10^4	Diabetes
59	53	F	Married	No	Outpatient	> 100	12×10^4	
60	52	F	Married	No	Outpatient	> 100	11×10^4	
61	53	F	Married	No	Outpatient	> 100	12×10^4	
62	63	M	Married		Outpatient	85	12×10^4	
63	57	F	Married	No	Outpatient	50	11×10^4	
64	60	F	Married	No	Inpatient	80	12×10^4	
65	49	M	Married		Inpatient	60	10×10^4	
66	48	M	Married		Inpatient	90	12×10^4	
67	55	M	Married		Inpatient	60	10×10^4	
68	72	M	Married		Inpatient	60	10×10^4	

69	53	M	Married		Inpatient	60	10 x 10^4	
70	55	M	Married		Inpatient	45	10 x 10^4	
71	40	F	Married	No	Inpatient	> 100	12 x 10^4	
72	67	M	Married		Inpatient	85	10 x 10^4	
73	57	M	Married		Inpatient	> 100	12 x 10^4	Catheter
74	59	M	Married		Inpatient	> 100	11 x 10^4	Catheter
75	59	M	Married		Inpatient	95	12 x 10^4	
76	59	M	Married		Inpatient	90	10 x 10^4	
77	57	M	Married		Inpatient	50	10 x 10^4	
78	56	M	Married		Inpatient	80	12 x 10^4	
79	**45**	M	Married		Inpatient	> 100	12 x 10^4	Catheter
80	**65**	M	Married		Inpatient	45	10 x 10^4	
81	**45**	M	Married		Inpatient	65	12 x 10^4	
82	**70**	M	Married		Inpatient	75	10 x 10^4	
83	60	F	Married	No	Inpatient	> 100	11 x 10^4	
84	**66**	M	Married		Inpatient	80	10 x 10^4	
85	**53**	M	Married		Inpatient	> 100	14 x 10^4	Catheter
86	**70**	M	Married		Inpatient	> 100	12 x 10^4	Catheter
87	57	F	Married	No	Inpatient	> 100	11 x 10^4	
88	**75**	M	Married		Inpatient	> 100	14 x 10^4	Catheter
89	**59**	M	Married		Inpatient	> 100	10 x 10^4	
90	**72**	M	Married		Inpatient	50	10 x 10^4	
91	**42**	M	Married		Inpatient	> 100	11 x 10^4	Catheter
92	**51**	M	Married		Inpatient	> 100	12 x 10^4	Catheter
93	**40**	M	Married		Inpatient	70	10 x 10^4	
94	**49**	M	Married		Inpatient	80	10 x 10^4	
95	**61**	M	Married		Inpatient	65	10 x 10^4	
96	**62**	M	Married		Inpatient	> 100	14 x 10^4	Catheter
97	**63**	M	Married		Inpatient	80	12 x 10^4	
98	**63**	M	Married		Inpatient	80	12 x 10^4	
99	59	F	Married	No	Inpatient	> 100	14 x 10^4	Catheter
100	57	F	Married	No	Inpatient	50	11 x 10^4	

M = Male
F = Female
Inpatient = Hospital acquired infection
Outpatient = Community acquired infection
K.S = Kidney stones
HPF= High power field
CFU = Colony forming unit

Table (2): Urine samples collected from different ages of males and females patients.

Age of samples	Total urine samples	Positive urine samples	Percentage%	Personal state		Pregnant	
				Single	Married	Yes	No
Age of females (years)							
20-25	16	12	75.00	3	13	3	13
26-30	13	10	76.92	1	12	6	7
31-35	6	2	33.33	2	4	2	4
36-40	3	2	66.66	-	3	1	2
41-45	5	3	60.00	2	3	-	5
46-50	4	3	75.00	-	4	-	4
51-55	7	6	85.71	-	7	-	7
56-60	11	9	81.81	-	11	-	11
20-60	65	47	72.30	8	57	12	53
Age of males (years)							
35-40	5	3	60.00	1	4		
41-45	6	5	83.33	3	3		
46-50	8	6	75.00	-	8		
51-55	8	5	62.50	-	8		
56-60	23	16	69.56	-	23		
61-65	12	9	75.00	-	12		
66-70	5	4	80.00	-	5		
71-75	6	5	83.33	-	6		
35-75	73	53	72.60	4	69		
Total	138	100	72.46	12	126	12	53

$$\% \text{ Positive} = \frac{\text{Positive samples}}{\text{Total samples}} \times 100$$

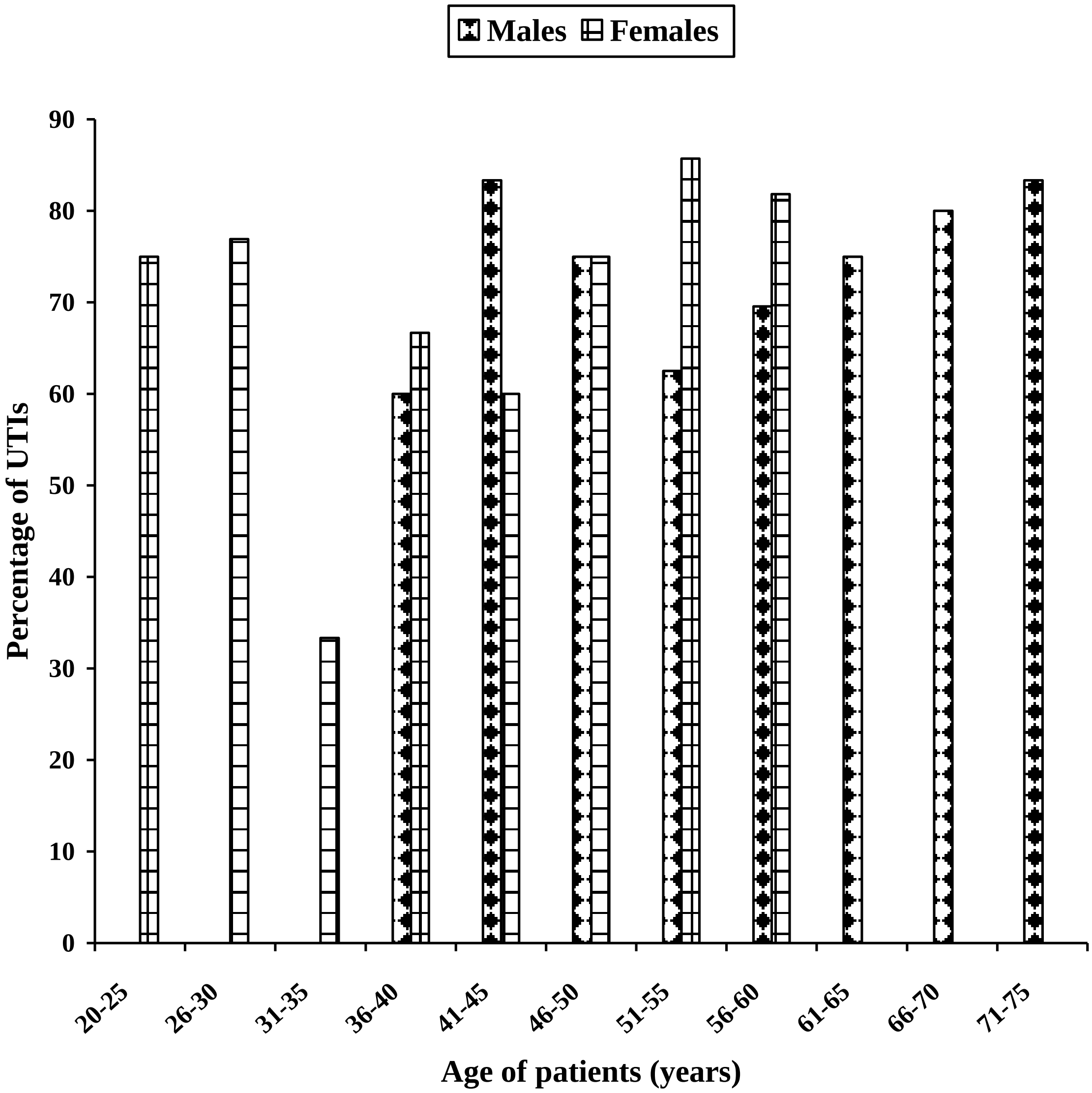


Fig. (1): Urine samples collected from patients with different ages of males and females.

Table (3): Statistical analysis and characteristics of the studied groups.

Characters	Positive samples		Negative samples		Test of significant	P
Age					T	
$\overline{X}$ ± SD	48.7±15.6		45.4±14.5		1.12	0.26
Range	20-75		20-71			NS
Gender	No	%	No	%	X^2	P
Males	53	53.00	20	52.63	0.001	0.96 NS
Females	47	47.00	18	47.37		
Personal states						
Single	8	8.00	4	10.53	0.02	0.89 NS
Married	92	92.00	34	89.47		
Pregnant (female)						
Yes	9	19.15	3	16.67	0.02	0.89 NS
No	38	80.85	15	83.33		

No. = Number
$\overline{X}$ = Arithmetic mean
SD = Standard deviation
X^2 = Test of significance (Chi – square)
T = Test of significant
P = Probability
P > 0.05 = Non significant
P < 0.05 = Significant
P < 0.01 = Highly significant
NS = Non significant
S = Significant
HS = Highly significant

2- Community-acquired and hospital-acquired UTIs.

Urinary tract infections (UTIs) remain the common infections diagnosed in outpatients (community-acquired) as well as inpatients (hospitalized patients).

The obtained results in table (4) and figure (2) clearly demonstrated that the highest percentage of community-acquired infection obtained was 63%, while the percentage of hospital-acquired infection was 37%.

In community-acquired infection the percentage of females (65.1%) were more than the percentage of males (34.9%), on the contrary in the hospital-acquired infection the percentage of male (83.78%) were more than the percentage of females (16.22%), the statistical analysis for these results were performed according to community-acquired infection, and hospital-acquired infection and these results were highly significant.

Table (4): Community-acquired and hospital-acquired UTIs.

	No. of patients	Percentage %	Males	Percentage %	Females	Percentage %	X^2	P
Community -acquired UTIs	63	63.00	22	34.9	41	65.1	22.34	<0.001 HS
Hospital -acquired UTIs	37	37.00	31	83.78	6	16.22		
Total - acquired UTIs	100	100.00	53	53.00	47	47.00		

Community acquired infection = Outpatients
Hospital acquired infection = Inpatients

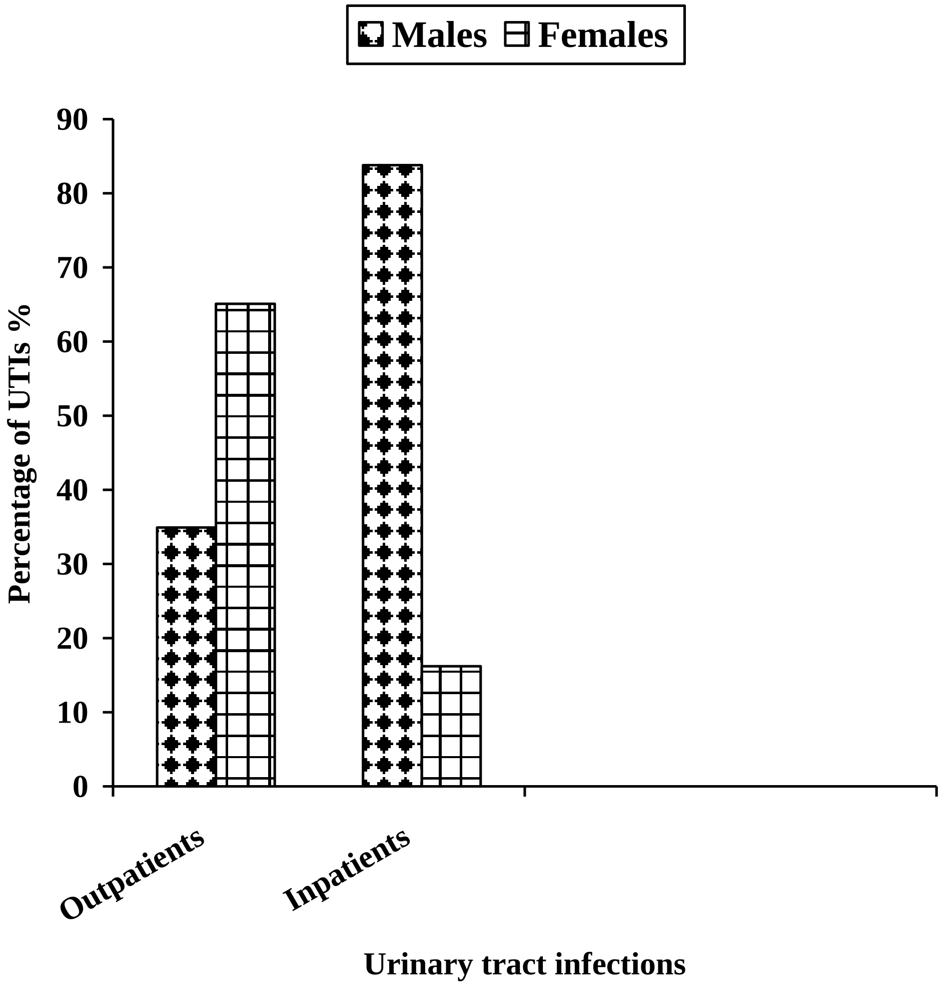


Fig. (2): Community-acquired and hospital -acquired UTIs.

3- Specific risk factors complicated urinary tract infections.

The specific risk factors complicated urinary tract infections as diabetes, kidney stones and catheterization were studies in this experiment to illustrate that, there is a strong relation between these risks and urinary tract infections of males and females samples.

The percentages of specific risk factors causing UTIs were 26.o%; it was distributed into diabetes (7.0%), kidney stones (10.0%) and catheterization (9.0%).

The percentages of specific risk factors in males were more than female in all cases of risk factors were recorded in diabetes, kidney stones and catheterization, 71.43 & 28.57, 60.00 & 40.00, 88.89 & 11.11% respectively.

The statistical analysis for these results were performed, where the results were significant incase catheterization and the results were non-significant incase diabetes and kidney stones as illustrated in table (5) and fig. (3).

Table (5): Specific risk factors causing urinary tract infections.

Specific risk factors	No. of patients	Percentage %	Males	Percentage %	Females	Percentage %	X^2	P
Diabetes	7	7.00	5	71.43	2	28.57	0.38	0.53 NS
Kidney stones	10	10.00	6	60.00	4	40.00	0.02	0.89 NS
Catheterization	9	9.00	8	88.89	1	11.11	5.11	0.02 S
Total	26	26.00	19	73.1	7	26.9		

$$\% \text{ Percentage of total} = \frac{\text{Total samples of risk factors}}{\text{Total positive urine samples}} \times 100$$

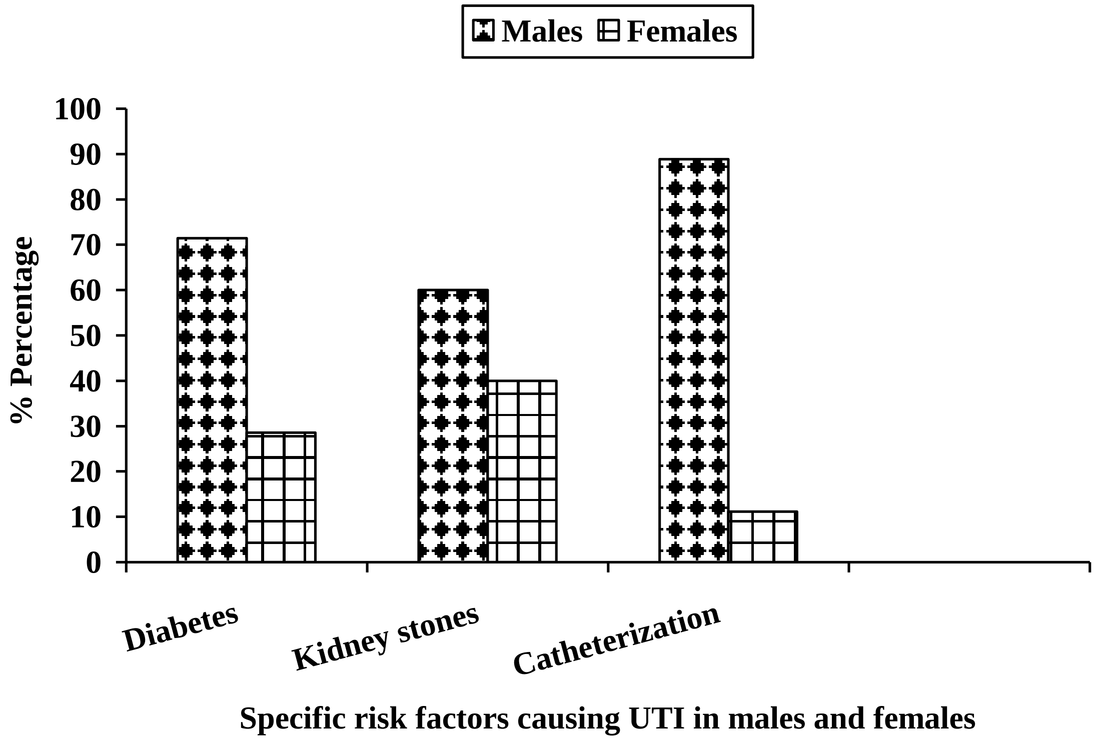


Fig. (3): Specific risk factors causing urinary tract infections.

4- The pus cells number and total count number of bacteria in males & females in positive urine samples.

The pus cells counts and total count number of bacteria per each milliliter of infected positive urine samples were counted as good indicators for urinary tract infection in males and females positive urine samples.

The obtained results in table (6) and fig. (5), illustrated that 23 positive male urine samples have pus cells number over than 100 hpf, while in female only 13 female patients have pus cells number over than 100 hpf.

Thirty three of positive patient urine samples have total bacterial count equal 10^{5}, where 13 of them were male with percentage 39.39% and 20 of them were female with percentage 60.61%, as illustrated in table (7) and fig. (6).

In general, the statistical analysis for these results were performed, where these results were significant incase pus cells number in positive urine samples and the results were non-significant incase total count number of bacteria per each positive urine samples.

Table (6): The pus cells number in positive samples of urine.

Pus cells No. /ml.	No. of samples	Males	Percentage %	Females	Percentage %
40-60	38	14	36.84	24	63.16
61-80	20	11	55.00	9	45.00
81-100	6	5	83.33	1	16.67
> 100	36	23	63.89	13	36.11
Total	100	53	53.00	47	47.00

X^2 = 7.94 **P = 0.047 S**

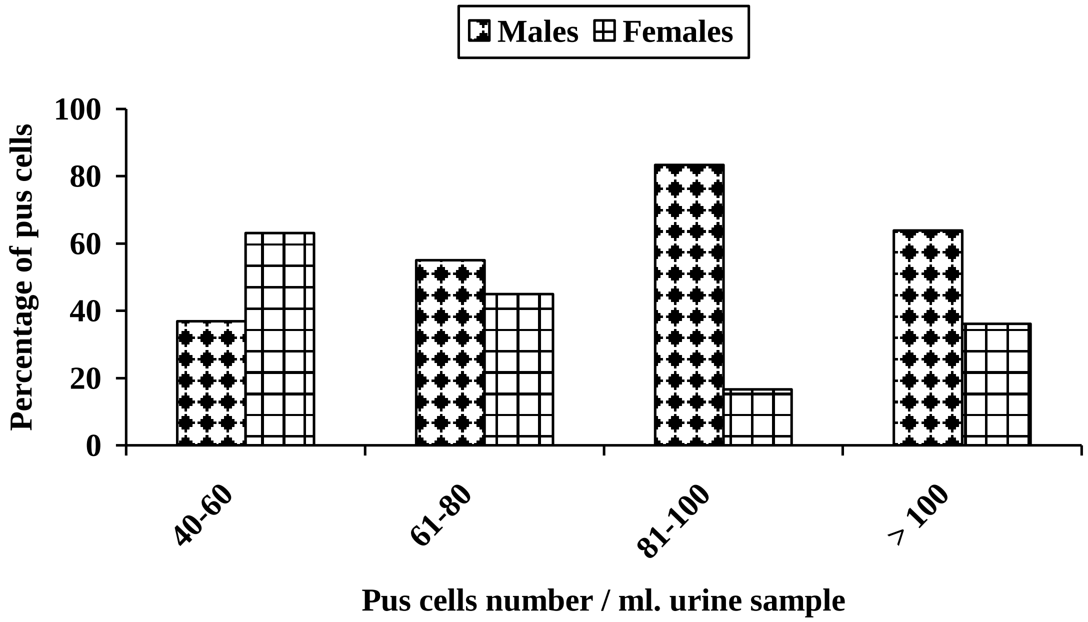


Fig. (4): The pus cells number in male and female in positive samples of urine.

Table (7): The total bacterial count (cfu/ml) in positive samples of urine.

Total bacterial count No. / ml	No. of patients	Males	Percentage %	Females	Percentage %
10×10^4	33	13	39.39	20	60.61
11×10^4	25	8	32.00	17	68.00
12×10^4	28	18	64.28	10	35.72
13×10^4	4	1	25.00	3	75.00
14×10^4	6	4	66.67	2	33.33
15×10^4	2	0.0	00.00	2	100.00
16×10^4	2	1	50.00	1	50.00
Total	100	53	53.00	47	47.00

$X^2 = 9.77$ P = 0.13 NS

$$\text{Total percentage \%} = \frac{\text{Total male or female samples}}{\text{Total positive urine samples}} \times 100$$

$$\text{\% Percentage of male or female} = \frac{\text{Male or female samples}}{\text{No. of positive samples}} \times 100$$

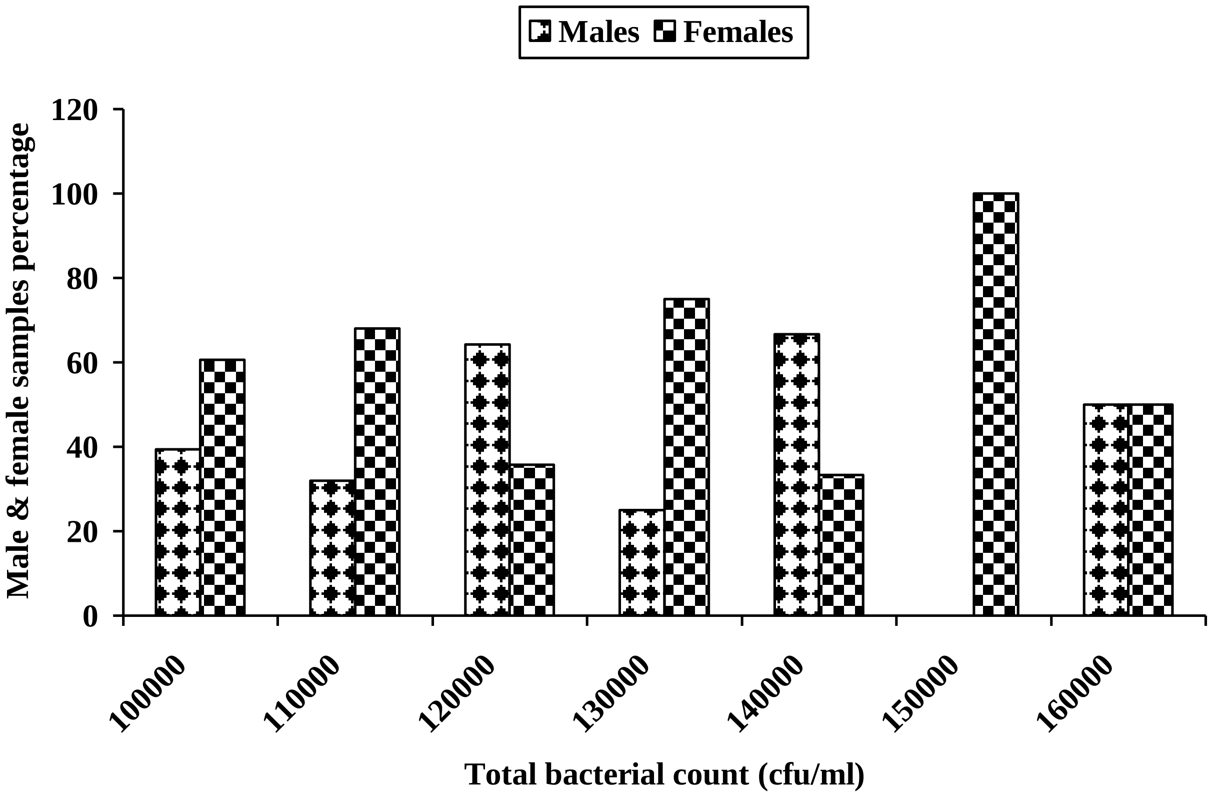


Fig. (5): The total bacterial count in positive samples of urine in males and females.

5- Identification of bacterial isolates.

The clinical isolates were subjected to two patterns of identification according to the Bergey's Manual of Determinative Bacteriology **(Holt *et al*, 1994),** as illustrated in table (8) and photos No. (1).

Firstly the staining reactions were conducted and the second is culture characteristics of isolates on simple, enriched and selective media as well as biochemical reactions.

According to Gram stain methods, 87 isolates were Gram-negative bacilli, while 13 isolates were Gram-positive cocci.

I- Gram-negative bacilli (87 isolates)

1- The first group of Gram-negative bacilli isolates: includes 40 isolates

Colonial morphology: Circular, convex, smooths colonies with distinct edges.

Cultural properties: Facultative anaerobic, produce rose-pink to red colonies on MacConkey agar media and yellow colonies when cultured on CLED agar, so it is lactose fermentor. All isolates showed yellow slant and yellow butt (acidic for each) with gas bubbles in triple sugar iron agar (TSI) test, (A/A/g+/H_2S-).

Gram staining and microscopic examination: Gram-negative bacilli without special arrangement, non-capsulated and non spore-forming.

Biochemical characteristics: produce catalase enzyme, potassium hydroxide test positive, motile, produce indole, and give positive methyl-red reaction and negative Voges-Proskauer reaction, urease and citrate are negative; H_2S and oxidase test are negative.

Carbohydrates fermentation: Glucose, sucrose, maltose, lactose and mannitol are positive results.

According to the above characteristics the 40 isolates of the first group of Gram-negative bacilli were identified as strains belonging to *Escherichia coli*.

2- The second group of Gram-negative bacilli isolates: include 15 isolates.

Colonial morphology: Round, slightly large, undulate, unbenate and produce mucoid colonies on media rich with sugar like MacConkey agar media.

Cultural properties: Facultative anaerobic, produce rose-pink to red mucoid colonies on MacConkey agar media and yellow mucoid colonies when cultured on CLED agar, so it is lactose fermentor. All isolates showed yellow slant and yellow butt (acidic for each) with gas bubbles in triple sugar iron agar (TSI) test, (A/A/g+/H_2S-).

Gram stain and microscopic examination: Gram negative bacilli with rounded ends, non spore- forming, encapsulated.

Biochemical characteristics: Potassium hydroxide test positive, produce catalase enzyme, non-motile, indole test negative, give negative methyl-red reaction and positive Voges-Proskauer reaction, urease and citrate are positive, H_2S and oxidase test are negative.

Carbohydrates fermentation: Glucose, sucrose, maltose, lactose and mannitol are positive results.

According to the above characteristics the 15 isolates of the second group of Gram-negative bacilli were identified as strains belonging to *Klebsiella pneumoniae*.

3- The third group of Gram-negative bacilli isolates: include 22 isolates

Colonial morphology: Round, convex, small, with entire margin, tan and mucoid colonies.

Cultural properties: Aerobic and facultative anaerobic produce swarming growth on nutrient agar and blood agar, colorless colonies without swarming on MacConkey agar and CLED agar, so it is non-lactose fermentor. All isolates showed yellow slant and yellow-black butt (acidic for each) in triple sugar iron agar (TSI) test, (A/A/g-/H_2S+).

Gram staining and microscopic examination: Gram-negative bacilli, non-capsulated, pleomorphic and non spore-forming.

Biochemical characteristics: Potassium hydroxide test positive, produce catalase enzyme, motile, indole test negative, give positive methyl-red reaction and negative Voges-Proskauer reaction, urease and citrate are positive, H2S test are positive and oxidase test are negative.

Carbohydrates fermentation: Glucose is positive, lactose is negative, some strains are positive and some strains are negative for maltose, sucrose and mannitol.

According to the above characteristic the 22 isolates of the third group of Gram-negative bacilli were identified as strains belonging to *Proteus mirabilis*.

4- The fourth group of Gram-negative bacilli isolates: include 10 isolates

Colonial morphology: Flat, small and rough colonies, grayish-green to bluish colonies with mucoid texture and irregular margins.

Cultural properties: Strict aerobic, produce water soluble (diffusible) green, blue, or other color pigments which called diffusible pigments. Almost all of these isolates produced green diffusible pigment on nutrient agar, MacConkey agar and CLED agar, it is non-lactose fermentor. All isolates showed red slant and red butt (alkaline for each) in triple sugar iron agar (TSI) test, (K/K/g-/H_2S-).

Gram staining and microscopic examination: Gram-negative bacilli, straight rods, non-capsulated and non spore-forming.

Biochemical characteristics: Potassium hydroxide test positive, produce catalase enzyme, motile, indole test negative, give negative methyl-red reaction and negative Voges-Proskauer reaction, urease are negative and citrate are positive, H_2S test are negative and oxidase test are positive.

Carbohydrates fermentation: Glucose is positive incase oxidation only, lactose is negative, sucrose and mannitol are negative and maltose is positive.

According to the above characteristic the 10 isolates of the fourth group of Gram-negative bacilli were identified as strains belonging to *Pseudomonas aeruginosa*

II- Gram-positive cocci (13 isolates)

Gram positive cocci isolates: include 13 isolates

Colonial morphology: Smooth, convex, circular colonies with white color.

Cultural properties: Facultative anaerobic,

Gram staining and microscopic examination: Gram-positive cocci, arranged in clusters, non-capsulated and non spore-forming.

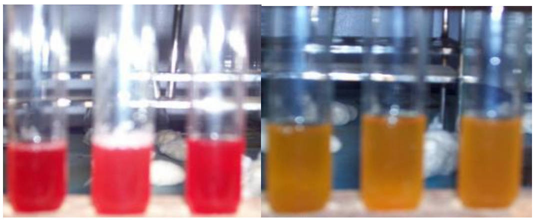
Biochemical characteristics: Potassium hydroxide test negative, produce catalase enzyme, non-motile, no growth on macConkey's media, non-coagulase, can grow on mannitol salt agar and also produce positive result, resistant to novobiocin antibiotics.

Carbohydrates fermentation: Glucose and mannitol are positive and the remaining sugars are negative results.

According to the above characteristic the 13 isolates of this group of Gram-positive cocci were identified as strains belonging to *Staphylococcus saprophyticus*.

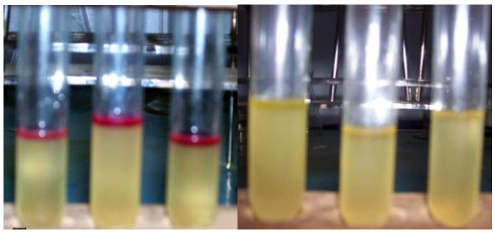
Table (8): Biochemical reaction of *E. coli*, *Klebsiella pneomoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus*.

Biochemical test	**Bacterial isolates**				
	E. coli	***K. pneomoniae***	***P. mirabilis***	***P. aeruginosa***	***S. saprophyticus***
Gram stain	-ve	-ve	-ve	-ve	+ve
KOH test	+ve	+ve	+ve	+ve	-ve
Catalse test	+ve	+ve	+ve	+ve	+ve
Oxidase test	-ve	-ve	-ve	+ve	
Lactose	+ve	+ve	-ve	-ve	
Glucose	+ve	+ve	+ve	+ve in O_2	+ve
Maltose	+ve	+ve	±ve	-ve	
Sucrose	+ve	+ve	±ve	-ve	
Mannitol	+ve	+ve	±ve	-ve	+ve
H_2S	-ve	-ve	+ve	-ve	
Indole test	+ve	-ve	-ve	-ve	
Methyl red	+ve	-ve	+ve	-ve	
Voges proskauer	-ve	+ve	-ve	-ve	
Citrate utilization	-ve	+ve	+ve	+ve	
Urease	-ve	+ve	+ve	-ve	
Nitrate reduction	+ve	+ve	+ve	+ve	
Motility	Motile	Non-motile	Motile	Motile	Non-motile
Coagulase test					-ve
Mannitol salt agar					+ve
Novobiocin antibiotic					Resistant



+ve -ve

Methyl red test



+ve -ve

Indole test

-ve +ve

Urease test

-ve +ve

Citrate test

+ve -ve

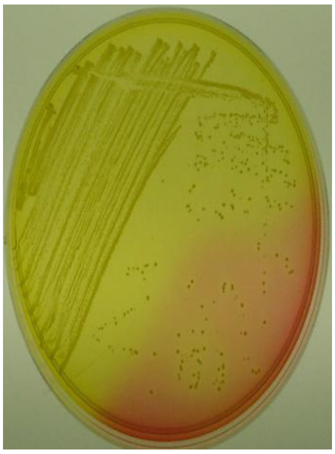
Coagulase test

-ve +ve

Nitrate reduction test

Control	(A/A/g+/H_2S-)	(A/A/g+/H_2S-)	(A/A/H_2S+)	(K/K/ H_2S-)

TSI test



S. saprophyticus on mannitol salt agar

S. saprophyticus on blood agar

Photos No. (1): Biochemical reaction used in identification of bacterial isolates.

6- The distribution of pathogenic isolate from positive urine samples.

The results in table (9) and fig. (6,7). Indicate that the number of contaminated positive urine samples collected from males and females were 100 samples, 40 contaminated with *E. coli*, 22 of *Proteus mirabilis*, 15 of *Klebsiella pneomoniae*, 13 of *Staphylococcus saprophyticus* and 10 of *Pseudomonase aeruginosa*.

So the highest percentage of distribution are found in *E. coli* (40%) followed by *Proteus mirabilis* (22%), *Klebsiella pneomoniae* (15%), *Staphylococcus saprophyticus* (13%) and *Pseudomonase aeruginosa* (10%).

The statistical analyses for these results are performed, where the results are highly significant.

Table (9): The distribution of pathogenic bacterial isolates among collected samples.

Pathogenic Bacterial isolates	Total samples	Positive samples	Distribution number				Percentage of distribution, (%)
			Male	%	Female	%	
E. coli	100	40	21	53.13	19	46.87	40.00
P. mirabilis		22	11	50.00	11	50.00	22.00
K. ***pneumoniae***		15	8	53.33	7	46.66	15.00
S. saprophyticus		13	9	69.23	4	30.77	13.00
P. aeruginosa		10	6	60.00	4	40.00	10.00

X^2 = 36.13 P <0.001 HS

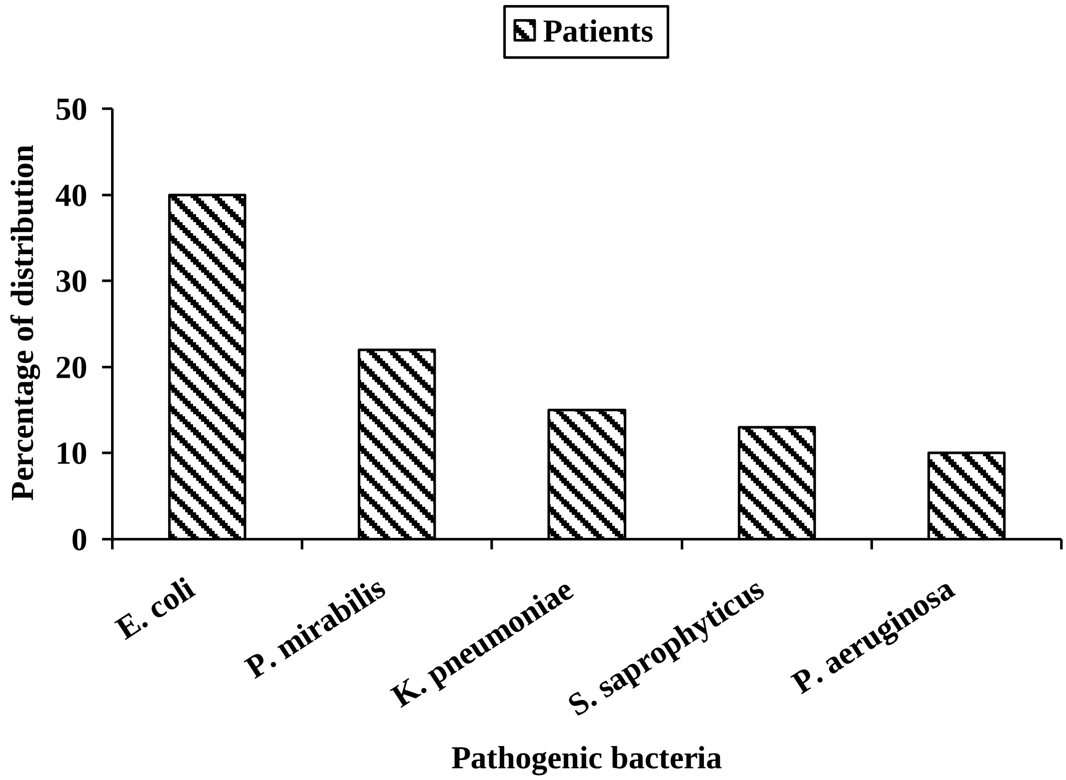


Fig. (6): The distribution number of pathogenic bacterial isolate from positive collected samples.

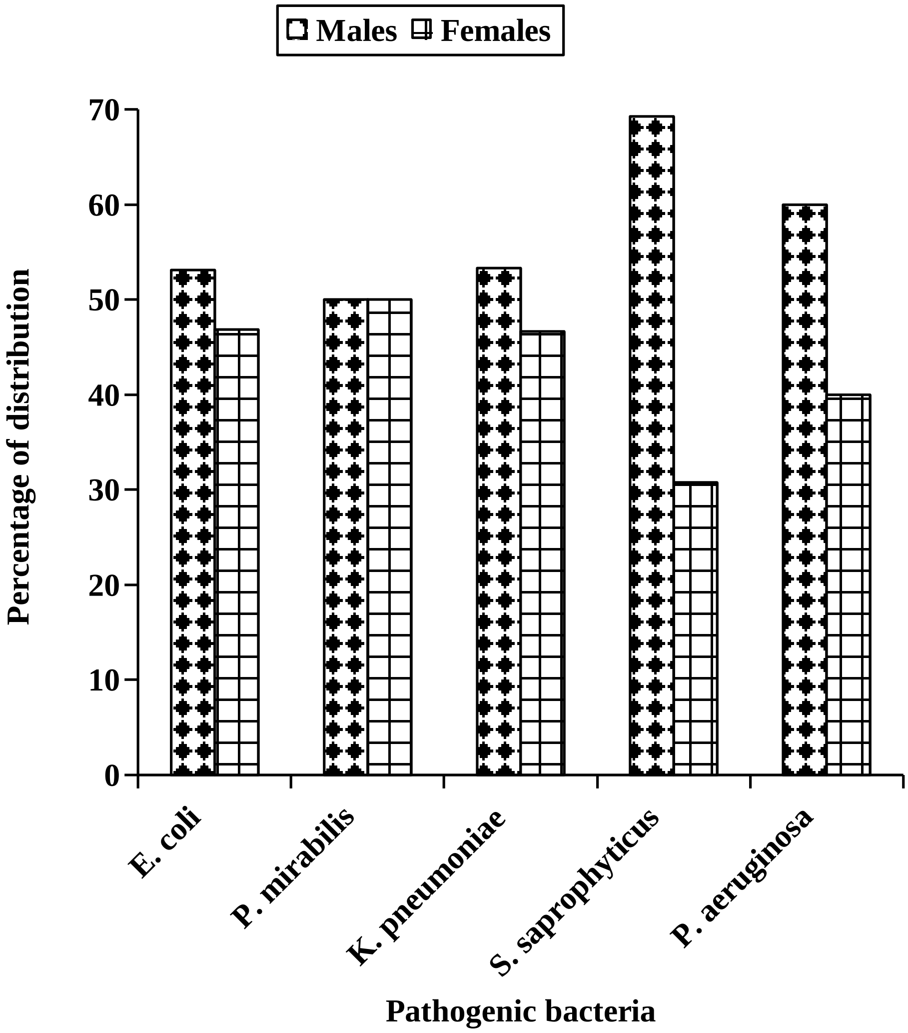


Fig (7): The distribution number of pathogenic bacterial isolates from collected positive samples of bacteria in males and females.

7- Susceptibility of pathogenic bacterial isolates to antibiotics drugs.

Different commercial antibiotics used to show their effect against pathogenic isolated bacterial organisms causing urinary tract infection. The antibiotics disc recommended concentration used were penicillin-G (P.G) (10µg), ampicillin (AM) (10µg), azithromycin (AZM) (15µg), norfloxacin (NOR) (10µg), ofloxacin (OFX) (5µg), amikacin (AK) (30µg), chloramphenicol (C) (30µg), cefotaxime sodium (CTX) (30µg), as illustrated in table (10) and Photos No. (2).

The obtained results clearly indicated that penicillin-G with (10µg /disc) not effective against all tested isolated pathogenic bacteria, followed by ampicillin with (10 µg/disc) concentration and cefotaxime sodium with (30 µg/disc) concentration which indicated a weak susceptibility effect on tested bacterial isolates.

The antibiotics ofloxacin with concentration (5 µg/disc) showed the highest effect against all tested pathogenic bacteria followed by amikacin with concentration (30 µg/disc) showed moderate effect followed by chloramphenicol, norfloxacin and azithromycin.

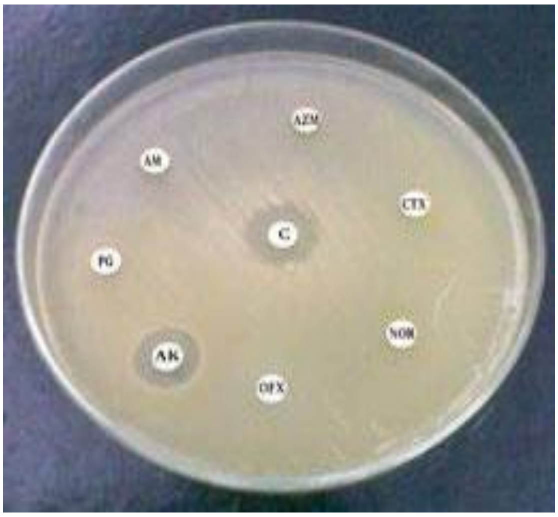
The pathogenic bacterial isolates *E. coli* number 69 & 72, *Proteus mirabilis* number 43 & 55 and *Pseudomonas aeruginosa* number 42 showed resistant against antibiotics penicillin-G, ampicillin, cefotaxim sodium, azithromycin, ofloxacin and norfloxacin, and *Klebsiella pneumoniae* number 93, *E. coli* number 24, *Proteus mirabilis* number 45 & 47 and *Pseudomonas aeruginosa* number 96 indicated the highest clinical resistance against antibiotics penicillin-G, ampicillin, cefotaxime sodium, azithromycin, ofloxacin, norfloxacin, amikacin and chloramphenicol.

Table (10): Inhibition zone (mm) of different pathogenic bacterial isolates against different tested antibiotics drugs.

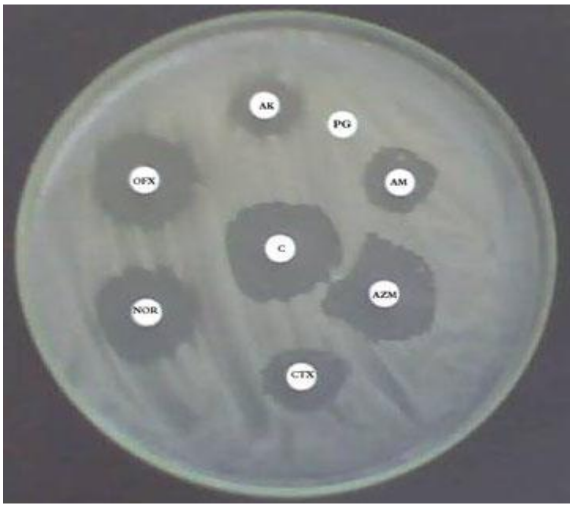
No.	Pathogeni bacterial isolates	Diameter of inhibition zone (mm) against different antibiotics discs.															
		NOR (10 µg)		CTX (30 µg)		AZM (15 µg)		OFX (5 µg)		AM (10 µg)		P.G (10 µg)		AK (30 µg)		C (30 µg)	
		IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST
1	*E. coli*	30	S	ND		21	S	33	S	ND		ND		14	R	25	S
2	*P. aeruginosa*	28	S	ND		16	I	24	S	ND		ND		22	S	ND	
3	*E. coli*	31	S	ND		15	I	30	S	25	S	ND		19	S	29	S
4	*K. pneumoniae*	28	S	18	I	20	S	30	S	ND		ND		20	S	25	S
5	*E. coli*	30	S	ND		25	S	32	S	ND		ND		20	S	30	S
6	*E. coli*	26	S	15	I	24	S	28	S	ND		ND		22	S	30	S
7	*E. coli*	30	S	25	I	15	I	30	S	28	S	ND		17	S	30	S
8	*K. pneumoniae*	30	S	10	R	10	R	33	S	ND		ND		18	S	30	S
9	*S. saprophyticus*	18	S	ND		25	S	30	S	25	R	ND		20	S	24	S
10	*K. pneumoniae*	30	S	11	R	18	S	20	S	9	R	ND		18	S	25	S
11	*E. coli*	38	S	22	I	25	S	27	S	ND		ND		20	S	30	S
12	*E. coli*	ND		ND		13	R	10	R	ND		ND		15	I	25	S
13	*E. coli*	35	S	ND		10	R	30	S	ND		ND		15	I	30	S
14	*E. coli*	35	S	ND		20	S	37	S	ND		ND		22	S	34	S
15	*E. coli*	27	S	ND		20	S	35	S	ND		ND		21	S	40	S
16	*E. coli*	28	S	17	I	16	I	30	S	ND		ND		17	S	ND	
17	*E. coli*	31	S	15	I	21	S	36	S	ND		ND		16	I	ND	
18	*E. coli*	ND		ND		ND		ND		ND		ND		24	S	ND	
19	*Proteus mirabilis*	23	S	9	R	15	I	24	S	ND		ND		20	S	10	R
20	*E. coli*	35	S	ND		20	S	20	S	ND		ND		21	S	33	S
21	*S. saprophyticus*	26	S	ND		22	S	34	S	ND		ND		22	S	24	S
22	*Proteus mirabilis*	35	S	ND		9	R	35	S	ND		ND		22	S	13	I
23	*E. coli*	ND		ND		18	S	ND		ND		ND		16	I	12	R
24	*E. coli*	ND		ND		ND		ND		ND		ND		11	R	ND	
25	*E. coli*	10	R	9	R	9	R	9	R	9	R	ND		18	S	22	S
26	*E. coli*	35	S	20	I	14	I	ND		ND		ND		15	I	ND	
27	*E. coli*	ND		ND		18	S	ND		ND		ND		16	I	9	R
28	*Proteus mirabilis*	ND		ND		30	S	ND		ND		ND		20	S	ND	
29	*P. aeruginosa*	13	I	ND		30	S	15	I	24	S	ND		16	I	30	S
30	*E. coli*	35	S	22	I	19	S	35	S	30	S	ND		16	I	30	S
31	*E. coli*	15	I	9	R	9	R	11	R	ND		ND		15	I	13	I
32	*S. saprophyticus*	27	S	ND		25	S	30	S	11	R	ND		20	S	27	S
33	*K. pneumoniae*	ND		ND		20	S	15	I	ND		ND		20	S	ND	
34	*P. aeruginosa*	ND		ND		17	I	17	S	16	I	ND		20	S	12	R
35	*P. aeruginosa*	18	S	ND		ND		20	S	ND		ND		19	S	ND	
36	*P. aeruginosa*	18	S	ND		ND		18	S	ND		ND		16	I	18	5
37	*Proteus mirabilis*	ND		ND		35	S	ND		ND		ND		10	R	ND	
38	*E. coli*	ND		ND		ND		ND		ND		ND		17	S	ND	
39	*K. pneumoniae*	ND		ND		30	S	ND		ND		ND		20	S	30	S
40	*S. saprophyticus*	15	I	9	R	25	S	20	S	28	R	ND		22	S	25	S
41	*E. coli*	ND		ND		ND		ND		9	R	ND		17	S	18	S
42	*P. aeruginosa*	ND		ND		ND		ND		ND		ND		15	I	ND	
43	*Proteus mirabilis*	ND		ND		12	R	10	R	ND		ND		15	I	10	R
44	*P. aeruginosa*	20	S	ND		ND		20	S	ND		ND		15	I	9	R
45	*Proteus mirabilis*	ND		ND		ND		ND		ND		ND		14	R	ND	
46	*Proteus mirabilis*	25	S	ND		ND		26	S	ND		ND		18	S	ND	

47	***Proteus mirabilis***	**ND**		**10**	**R**	**ND**		**ND**		**10**	**R**	**ND**		**14**	**R**	**ND**	
48	***K. pneumoniae***	**ND**		**ND**		**36**	**S**	**ND**		**ND**		**ND**		**20**	**S**	**26**	**S**
49	***E. coli***	**15**	**I**	**ND**		**ND**		**18**	**S**	**ND**		**ND**		**11**	**R**	**ND**	
50	***S. saprophyticus***	**10**	**R**	**ND**		**15**	**I**	**30**	**S**	**30**	**S**	**ND**		**24**	**S**	**20**	**S**
51	***K. pneumoniae***	**ND**		**ND**		**ND**		**ND**		**ND**		**ND**		**18**	**S**	**ND**	
52	***Proteus mirabilis***	**ND**		**ND**		**25**	**S**	**ND**		**ND**		**ND**		**17**	**S**	**ND**	
53	***K. pneumoniae***	**22**	**S**	**ND**		**12**	**R**	**20**	**S**	**ND**		**ND**		**18**	**S**	**28**	**S**
54	***Proteus mirabilis***	**25**	**S**	**ND**		**28**	**S**	**30**	**S**	**20**	**S**	**ND**		**20**	**S**	**30**	**S**
55	***Proteus mirabilis***	**ND**		**ND**		**ND**		**ND**		**ND**		**ND**		**15**	**I**	**ND**	
56	***E. coli***	**28**	**S**	**15**	**I**	**ND**		**28**	**S**	**26**	**S**	**ND**		**13**	**R**	**10**	**R**
57	***E. coli***	**ND**		**ND**		**13**	**R**	**ND**		**ND**		**ND**		**20**	**S**	**ND**	
58	***E. coli***	**32**	**S**	**ND**		**22**	**S**	**30**	**S**	**ND**		**ND**		**22**	**S**	**31**	**S**
59	***K. pneumoniae***	**32**	**S**	**21**	**I**	**21**	**S**	**35**	**S**	**ND**		**ND**		**17**	**S**	**32**	**S**
60	***Proteus mirabilis***	**18**	**S**	**ND**		**13**	**R**	**18**	**S**	**ND**		**ND**		**16**	**I**	**24**	**S**
61	***S. saprophyticus***	**ND**		**ND**		**20**	**S**	**22**	**S**	**17**	**R**	**ND**		**19**	**S**	**40**	**S**
62	***S. saprophyticus***	**20**	**S**	**16**	**I**	**35**	**S**	**20**	**S**	**35**	**S**	**ND**		**34**	**S**	**13**	**I**
63	***E. coli***	**12**	**R**	**ND**		**21**	**S**	**15**	**I**	**ND**		**ND**		**15**	**I**	**38**	**S**
64	***K. pneumoniae***	**ND**		**ND**		**24**	**S**	**ND**		**ND**		**ND**		**18**	**S**	**11**	**R**
65	***E. coli***	**ND**		**15**	**I**	**ND**		**ND**		**ND**		**ND**		**13**	**R**	**ND**	
66	***E. coli***	**28**	**S**	**ND**		**20**	**S**	**30**	**S**	**9**	**R**	**ND**		**12**	**R**	**28**	**S**
67	***E. coli***	**38**	**S**	**25**	**I**	**26**	**S**	**36**	**S**	**ND**		**ND**		**16**	**I**	**30**	**S**
68	***S. saprophyticus***	**22**	**S**	**ND**		**35**	**S**	**ND**		**ND**		**ND**		**22**	**S**	**35**	**S**
69	***E. coli***	**ND**		**ND**		**ND**		**ND**		**ND**		**ND**		**14**	**R**	**16**	**I**
70	***E. coli***	**ND**		**ND**		**ND**		**ND**		**27**	**S**	**ND**		**ND**		**30**	**S**
71	***S. saprophyticus***	**21**	**S**	**ND**		**32**	**S**	**20**	**S**	**ND**		**ND**		**25**	**S**	**30**	**S**
72	***E. coli***	**ND**		**ND**		**13**	**R**	**ND**		**ND**		**ND**		**14**	**R**	**13**	**I**
73	***P. aeruginosa***	**29**	**S**	**ND**		**11**	**R**	**25**	**S**	**ND**		**ND**		**20**	**S**	**15**	**I**
74	***Proteus mirabilis***	**ND**		**ND**		**25**	**S**	**ND**		**ND**		**ND**		**15**	**I**	**ND**	
75	***S. saprophyticus***	**28**	**S**	**ND**		**20**	**S**	**30**	**S**	**ND**		**ND**		**21**	**S**	**21**	**S**
76	***S. saprophyticus***	**29**	**S**	**14**	**I**	**34**	**S**	**30**	**S**	**25**	**R**	**ND**		**27**	**S**	**30**	**S**
77	***K. pneumoniae***	**ND**		**ND**		**ND**		**14**	**I**	**ND**		**ND**		**15**	**I**	**ND**	
78	***S. saprophyticus***	**21**	**S**	**10**	**R**	**31**	**S**	**30**	**S**	**ND**		**ND**		**19**	**S**	**28**	**S**
79	***Proteus mirabilis***	**10**	**R**	**ND**		**10**	**R**	**20**	**S**	**ND**		**ND**		**15**	**I**	**9**	**R**
80	***K. pneumoniae***	**28**	**S**	**ND**		**15**	**I**	**28**	**S**	**ND**		**ND**		**16**	**I**	**ND**	
81	***E. coli***	**ND**		**ND**		**20**	**S**	**10**	**R**	**ND**		**ND**		**13**	**R**	**10**	**R**
82	***E. coli***	**ND**		**ND**		**ND**		**16**	**S**	**ND**		**ND**		**18**	**S**	**25**	**S**
83	***K. pneumoniae***	**30**	**S**	**ND**		**ND**		**ND**		**ND**		**ND**		**15**	**I**	**20**	**S**
84	***S. saprophyticus***	**28**	**S**	**ND**		**20**	**S**	**25**	**S**	**ND**		**ND**		**20**	**S**	**24**	**S**
85	***Proteus mirabilis***	**30**	**S**	**ND**		**28**	**S**	**32**	**S**	**22**	**S**	**ND**		**21**	**S**	**30**	**S**
86	***Proteus mirabilis***	**33**	**S**	**ND**		**19**	**S**	**40**	**S**	**ND**		**ND**		**14**	**R**	**13**	**I**
87	***E. coli***	**17**	**S**	**ND**		**30**	**S**	**16**	**S**	**ND**		**ND**		**15**	**I**	**15**	**I**
88	***P. aeruginosa***	**ND**		**ND**		**15**	**I**	**ND**		**ND**		**ND**		**14**	**R**	**14**	**I**
89	***Proteus mirabilis***	**10**	**R**	**ND**		**11**	**R**	**16**	**S**	**ND**		**ND**		**15**	**I**	**23**	**S**
90	***Proteus mirabilis***	**30**	**S**	**ND**		**14**	**I**	**27**	**S**	**ND**		**ND**		**16**	**I**	**30**	**S**
91	***Proteus mirabilis***	**15**	**I**	**ND**		**16**	**I**	**22**	**S**	**ND**		**ND**		**18**	**S**	**10**	**R**
92	***Proteus mirabilis***	**ND**		**9**	**R**	**13**	**R**	**ND**		**ND**		**ND**		**18**	**S**	**20**	**S**
93	***K. pneumoniae***	**ND**		**ND**		**ND**		**ND**		**ND**		**ND**		**9**	**R**	**ND**	
94	***K. pneumoniae***	**ND**		**ND**		**29**	**S**	**13**	**I**	**ND**		**ND**		**20**	**S**	**15**	**I**
95	***E. coli***	**ND**		**ND**		**20**	**S**	**ND**		**ND**		**ND**		**15**	**I**	**24**	**S**
96	***P. aeruginosa***	**ND**		**ND**		**ND**		**12**	**R**	**ND**		**ND**		**ND**		**ND**	
97	***Proteus mirabilis***	**20**	**S**	**ND**		**30**	**S**	**19**	**S**	**ND**		**ND**		**10**	**R**	**20**	**S**
98	***E. coli***	**25**	**S**	**ND**		**ND**		**25**	**S**	**ND**		**ND**		**14**	**R**	**35**	**S**
99	***Proteus mirabilis***	**ND**		**ND**		**25**	**S**	**ND**		**ND**		**ND**		**26**	**S**	**ND**	
100	***E. coli***	**ND**		**15**	**I**	**14**	**I**	**11**	**R**	**ND**		**ND**		**16**	**I**	**25**	**S**

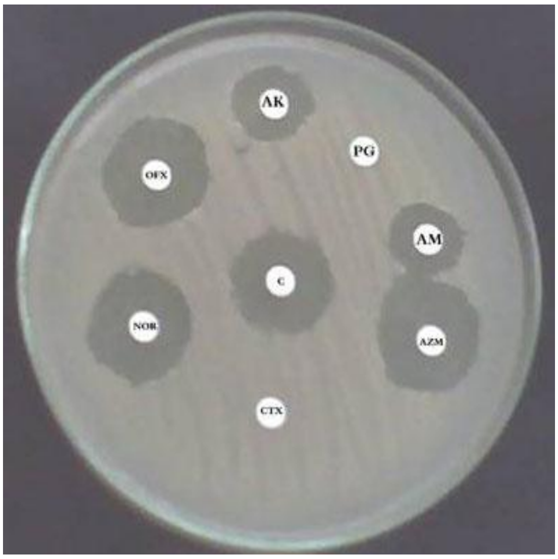
Susceptibility test = ST, Inhibition zone (mm) = IZ, Not detected (0.00) = ND.
Sensitive = S, Resistance = R, Intermediate = I.



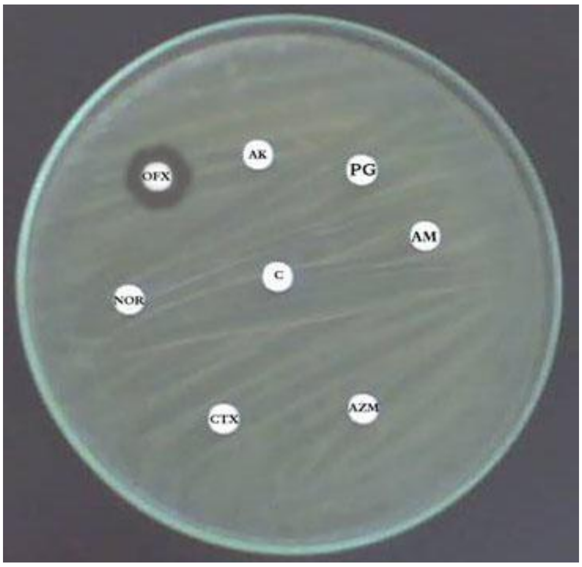
***E. coli* number 69**



***Staphylococcus saprophyticus* number 76**



***Proteus mirabilis* number 85**



***Pseudomonas aeruginosa* number 96**

Photos No. (2): Antibiotic susceptibility of bacterial isolates by disc diffusion method.

AK = amikacin; PG = penicillin-G; CTX = cefotaxime sodium; OFX = ofloxacin; NOR = norfloxacin; AM = ampicillin; AZE = azithromycin & C = chloramphenicol.

8- Sensitivity test of pathogenic isolates against different tested antibiotics drugs.

The sensitivity tests of pathogenic bacterial isolates against different tested antibiotics drugs were illustrated in table (11) and fig. (8).

These results showed that the antibiotic ofloxacin is more effective against isolated pathogenic bacterial organisms, which the percentage of sensitive organism reach to 58.0% followed by amikacin 54.0%, chloramphenicol 52.0%, norfloxacin 51.0%, azithromycin 48.0%, ampicillin 11.0%, cefotaxime sodium 2.0% and penicillin-G 0.0%.

The antibiotic amikacin show intermediate effect against isolated pathogenic bacterial organisms, which the percentage of organism reach to 27.0% followed by azithromycin 13.0%, cefotaxime sodium 12.0%, chloramphenicol 10.0%, ofloxacin 7.0%, norfloxacin 5.0%, ampicillin 1.0% and penicillin-G 0.0%.

On the other hand, the antibiotic penicillin-G hasn't any effect against isolated pathogenic bacterial organisms, which the percentage of resistance organism reach to 100.0% followed by ampicillin 88.0%, cefotaxime sodium 86.0%, norfloxacin 44.0%, azithromycin 39.0%, chloramphenicol 38.0%, ofloxacin 35.0% and amikacin 19.0%.

Table (11): Sensitivity test of pathogenic isolates against different antibiotics.

Tested antibiotics	Sensitive isolates		Intermediate isolates		Resistance isolates	
	No.	**%**	**No.**	**%**	**No.**	**%**
Norfloxacin (NOR)	51	51.0	5	5.0	44	44.0
Cefotaxime sodium(CTX)	2	2.0	12	12.0	86	86.0
Azithromycin (AZE)	48	48.0	13	13.0	39	39.0
Ofloxacin (OFX)	58	58.0	7	7.0	35	35.0
Ampicillin (AM)	11	11.0	1	1.0	88	88.0
Penicillin-G (PG)	0	0.0	0	0.0	100	100.0
Amikacin (AK)	54	54.0	27	27 .0	19	19.0
Chloramphenicol (C)	52	51.0	10	10.0	38	38.0

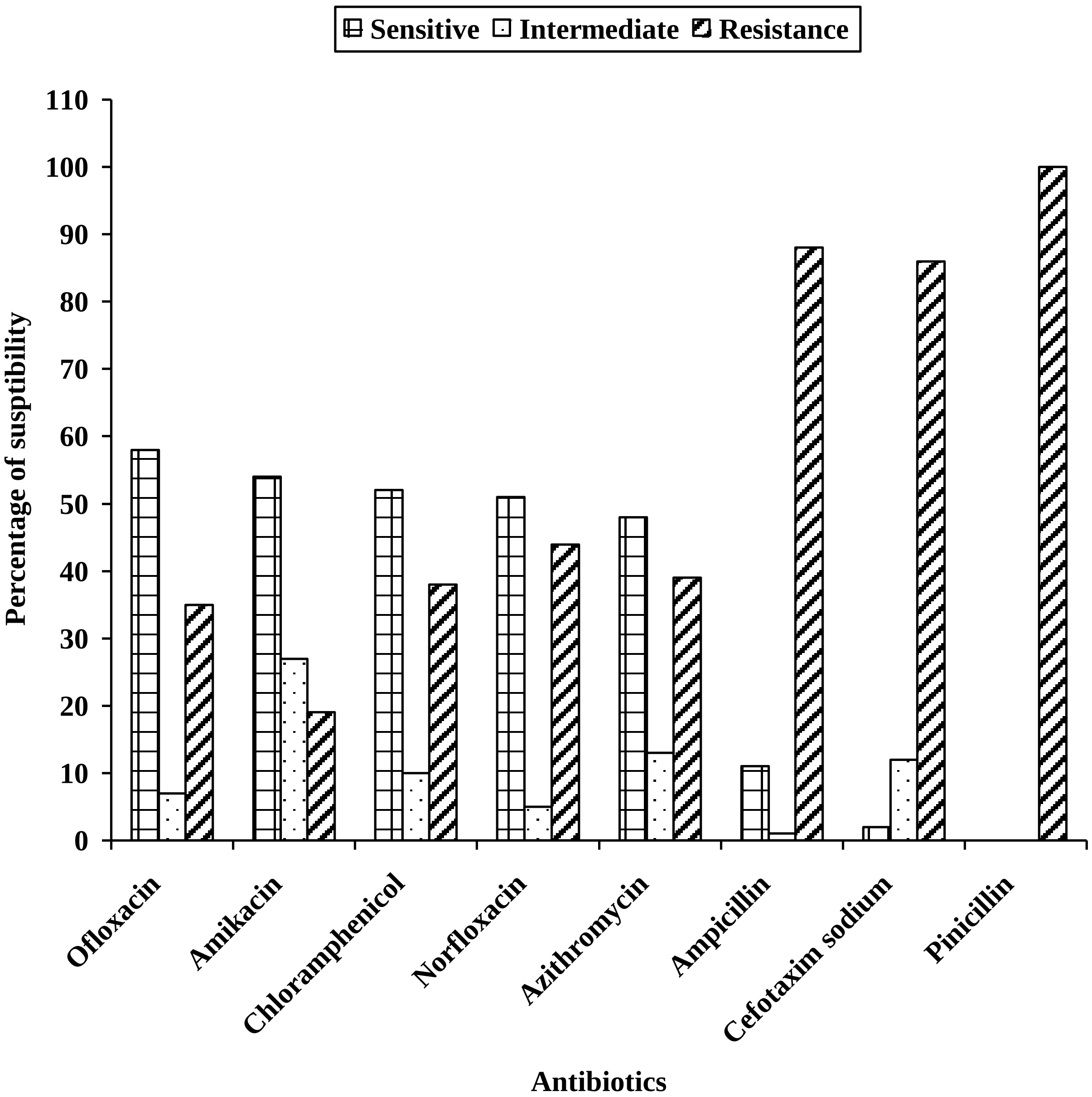


Fig. (8): The sensitivity test of pathogenic isolates against different antibiotics drugs.

9- Statistical analysis of sensitive, intermediate and resistant pathogenic bacterial isolates against different antibiotics.

The obtained results in table (12 a, b, c, d, e, f, g and h) showed that the sensitivity of pathogenic bacterial isolates against each tested antibiotic to illustrate the obtained results are significant or not.

The statistical analysis incase amikacin and penicillin-G are highly significant, while incase ofloxacin, chloramphenicol, norfloxacin, azithromycin and cefotaxime sodium are significant, but incase ampicillin, it is non-significant.

These results clearly indicated that *E. coli* showed highly resistant against all tested antibiotics drugs which recorded the percentage of resistant 47.4, 57.9, 34.2, 40.0, 40.9, 43.6 and 39.4 against ofloxacin, amikacin, chloramphenicol, cefotaxime sodium, penicillin-G, norfloxacin, azithromycin and ampicillin respectively followed by *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus* showed the lowest resistance against tested antibiotics drugs. On the other hand the antibiotic ampicillin has the lowest effect against tested clinical bacterial isolates, cefotaxime sodium not affect against tested clinical bacterial isolates and penicillin-G recorded zero percent in sensitivity and intermediate against tested clinical bacterial isolates.

Table (12): Statistic analysis of sensitive, intermediate and resistance pathogenic bacterial isolates against different antibiotics.

a) Antibiotic ofloxacin (OFX)

Isolated organisms	Sensitive		Intermediate		Resistant		X^2	P
	No.	%	No.	%	No.	%		
E. coli	21	36.8	1	20.0	18	47.4	**7.36**	**0.025 S**
P. mirabilis	12	21.1	0	0.0	10	26.3		
K. pneomoniae	6	10.5	3	60.0	6	15.8		
P. aeruginosa	6	10.5	1	20.0	3	7.9		
S. saprophyticus	12	21.1	0	0.0	1	2.6		

b) Antibiotic amikacin (AK)

Isolated organisms	Sensitive		Intermediate		Resistant		X^2	P
	No.	%	No.	%	No.	%		
E. coli	16	29.6	13	48.2	11	57.9	**21.42**	**<0.001 HS**
P. mirabilis	10	18.5	7	25.9	5	26.3		
K. pneomoniae	11	20.4	3	11.1	1	5.3		
P. aeruginosa	4	7.4	4	14.8	2	10.5		
S. saprophyticus	13	24.1	0	0.0	0	0.0		

c) Antibiotic chloramphenicol (C)

Isolated organisms	Sensitive		Intermediate		Resistant		X^2	P
	No.	%	No.	%	No.	%		
E. coli	23	44.2	4	40.0	13	34.2	**18.37**	**0.018 S**
P. mirabilis	7	13.5	2	20.0	13	34.2		
K. pneomoniae	8	15.4	1	10.0	6	15.8		
P. aeruginosa	2	3.8	2	20.0	6	15.8		
S. saprophyticus	12	23.1	1	10.0	0	0.0		

d) Antibiotic norfloxacin (NOR)

Isolated organisms	Sensitive		Intermediate		Resistant		X^2	P
	No.	%	No.	%	No.	%		
E. coli	20	39.3	2	40.0	18	40.9	**5.92**	**0.2 NS**
P. mirabilis	9	17.6	1	20.0	12	27.3		
K. pneomoniae	7	13.7	0	0.0	8	18.2		
P. aeruginosa	5	9.8	1	20.0	4	9.1		
S. saprophyticus	10	19.6	1	20.0	2	4.5		

e) Antibiotic azithromycin (AZM)

Isolated organisms	Sensitive		Intermediate		Resistant		X^2	P
	No.	%	No.	%	No.	%		
E. coli	18	37.5	5	38.4	17	43.6	**10.76**	**0.029 S**
P. mirabilis	9	18.7	3	23.1	10	25.6		
K. pneomoniae	8	16.7	1	7.7	6	15.4		
P. aeruginosa	1	2.1	3	23.1	6	15.4		
S. saprophyticus	12	25.0	1	7.7	0	0.0		

f) Antibiotic ampicillin (AM)

Isolated organisms	Sensitive		Intermediate		Resistant		X^2	P
	No.	%	No.	%	No.	%		
E. coli	5	50	0	0.0	35	39.4	**2.38**	**0.66 NS**
P. mirabilis	2	20	0	0.0	20	22.5		
K. pneomoniae	0	0.0	0	0.0	15	16.8		
P. aeruginosa	1	10	1	100.0	8	8.9		
S. saprophyticus	2	20	0	0.0	11	12.4		

g) Antibiotic cefotaxime sodium

Isolated organisms	Sensitive		Intermediate		Resistant		X^2	P
	No.	%	No.	%	No.	%		
E. coli	0	0.0	11	73.4	29	34.2	**10.58**	**0.03 S**
P. mirabilis	0	0.0	0	0.0	22	25.9		
K. pneomoniae	0	0.0	2	13.3	13	15.3		
P. aeruginosa	0	0.0	0	0.0	10	11.7		
S. saprophyticus	0	0.0	2	13.3	11	12.9		

h) Antibiotic penicillin-G

Isolated organisms	Sensitive		Intermediate		Resistant		X^2	P
	No.	%	No.	%	No.	%		
E. coli	0	0.0	0	0.0	40	40.0	**36.13**	**<0.001 HS**
P. mirabilis	0	0.0	0	0.0	22	22.0		
K. pneomoniae	0	0.0	0	0.0	15	15.0		
P. aeruginosa	0	0.0	0	0.0	10	10.0		
S. saprophyticus	0	0.0	0	0.0	13	13.0		

10- Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of selected antibiotics.

The experimental clinical bacteria were grown under stress of different concentration of the selected antibiotics in nutrient broth and the MICs and the MBCs were determined, as illustrated in figures (9, 10).

The results in table (13, a) indicated that the maximum MBC were obtained at ofloxacin antibiotic which recorded 250 µg/ml against *P. aeruginosa* number 96 & 42, *K. pneumoniae* number 93 and *Proteus mirabilis* number 43 & 55, and the lowest MBC were obtained 31.25 µg/ml at *E. coli* number 7 and *Staphylococcus saprophyticus* number 76. MBC equal to MIC which recorded 250 µg/ml & 125 µg/ml of ofloxacin against *P. aeruginosa* number 42 & *E. coli* number 65 respectively.

The results in table (13, b) indicated that the maximum MBC were obtained at amikacin antibiotic which recorded 250 µg/ml against *E. coli* number 24, 65 & 72, *P. aeruginosa* number 42, *K. pneumoniae* number 93 and *Proteus mirabilis* number 47 & 55, and the lowest MBC were obtained 62.5 µg/ml at *E. coli* number 7 and *Staphylococcus saprophyticus* number 9 & 76. MBC equal to MIC which recorded 250 µg/ml of amikacin against *E. coli* number 24, *K. pneumoniae* number 93 followed by *P. aeruginosa* number 96, *Proteus mirabilis* number 43 & 45 and *E. coli* number 69 which recorded 125 µg/ml followed by *Staphylococcus saprophyticus* number 9 & 76 which recorded 62.5 µg/ml.

The results in table (13, c) indicated that the maximum MBC were obtained at chloramphenicol antibiotic which recorded 250 µg/ml against *E. coli* number 24 & 65, *P. aeruginosa* number 42 & 96, *K. pneumoniae* number 93 and *Proteus mirabilis* number 43, 45, 47 & 55, and the lowest MBC were obtained 31.25 µg/ml at *Staphylococcus saprophyticus* number 76. MBC equal to MIC which recorded 250 µg/ml of chloramphenicol against *K. pneumoniae* number 93 and *P. aeruginosa* number 96 followed by *Staphylococcus saprophyticus* number 76 which recorded 31.25 µg/ml.

The results in table (13, d) indicated that the maximum MBC were obtained at norfloxacin antibiotic which recorded 250 µg/ml against *E. coli* number 24, 69 & 72, *P. aeruginosa* number 42 & 96, *K. pneumoniae* number 93 and *Proteus mirabilis* number 43, 45 & 47 , and the lowest MBC were obtained 31.25 µg/ml at *Staphylococcus saprophyticus* number 9. MBC equal to MIC which recorded 250 µg/ml & 125 µg/ml of norfloxacin against *E. coli* number 69 & *E. coli* number 65 respectively.

The statistical analyses for selected antibiotics (MIC & MBC) are highly significant, as illustrated in table (13 a, b, c, d).

Table (13): Minimum inhibitory concentration (MICs) (µg/ml) and minimum bactericidal concentration (MBCs) of different antibiotics.

a) Antibiotic ofloxacin.

Bacterial isolates	**No.**	**(MIC) (µg/ml)**	**(MBC) (µg /ml)**
E. coli	7	7.8125	31.25
S. saprophyticus	9	7.8125	62.5
E. coli	24	62.5	125
P. aeruginosa	42	250	250
P. mirabilis	43	125	250
P. mirabilis	45	62.5	125
P. mirabilis	47	62.5	125
P. mirabilis	55	125	250
E. coli	65	125	125
E. coli	69	62.5	125
E. coli	72	62.5	125
S. saprophyticus	76	7.8125	31.25
P. mirabilis	85	15.625	62.5
K. pneomoniae	93	125	250
P. aeruginosa	96	125	250
$\bar{X}$ **±SD**		**84.3±62.7**	**181.2±90.1**
t		**5.237**	
P		**0.001 HS**	

b) Antibiotic amikacin.

Bacterial isolates	No.	(MIC) (µg /ml)	(MBC) (µg /ml)
E. coli	7	31.25	62.5
S. saprophyticus	9	62.5	62.5
E. coli	24	250	250
P. aeruginosa	42	125	250
P. mirabilis	43	125	125
P. mirabilis	45	125	125
P. mirabilis	47	125	250
P. mirabilis	55	125	250
E. coli	65	125	250
E. coli	69	125	125
E. coli	72	125	250
S. saprophyticus	76	62.5	62.5
P. mirabilis	85	62.5	125
K. pneomoniae	93	250	250
P. aeruginosa	96	125	125
$\bar{X}$ **±SD**		**122.9±60.7**	**170.8±80.0**
t		**3.146**	
P		**0.007 HS**	

c) Antibiotic chloramphenicol.

Bacterial isolates	**No.**	**(MIC) (µg /ml)**	**(MBC) (µg /ml)**
E. coli	7	31.25	62.5
S. saprophyticus	9	31.25	62.5
E. coli	24	125	250
P. aeruginosa	42	125	250
P. mirabilis	43	62.5	250
P. mirabilis	45	125	250
P. mirabilis	47	125	250
P. mirabilis	55	125	250
E. coli	65	125	250
E. coli	69	62.5	125
E. coli	72	62.5	125
S. saprophyticus	76	31.25	31.25
P. mirabilis	85	31.25	125
K. pneomoniae	93	250	250
P. aeruginosa	96	250	250
$\bar{X}$ **±SD**		**104±71.5**	**185.4±85.5**
t		**5.348**	
P		**0.001 HS**	

d) Antibiotic norfloxacin.

Bacterial isolates	**No.**	**(MIC) (µg /ml)**	**(MBC) (µg /ml)**
E. coli	7	15.625	62.5
S. saprophyticus	9	15.625	31.25
E. coli	24	125	250
P. aeruginosa	42	125	250
P. mirabilis	43	62.5	250
P. mirabilis	45	62.5	250
P. mirabilis	47	125	250
P. mirabilis	55	62.5	125
E. coli	65	125	125
E. coli	69	250	250
E. coli	72	62.5	250
S. saprophyticus	76	15.625	62.5
P. mirabilis	85	31.25	62.5
K. pneomoniae	93	62.5	250
P. aeruginosa	96	125	250
$\bar{X}$ **±SD**		**81.7±65.6**	**145.8±83.2**
t		**5.682**	
P		**0.001 HS**	

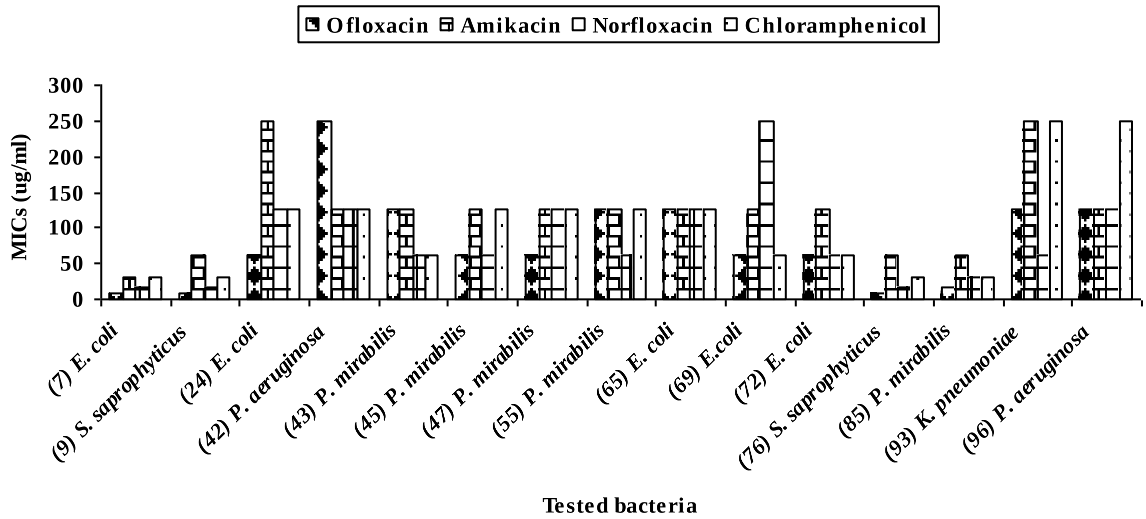
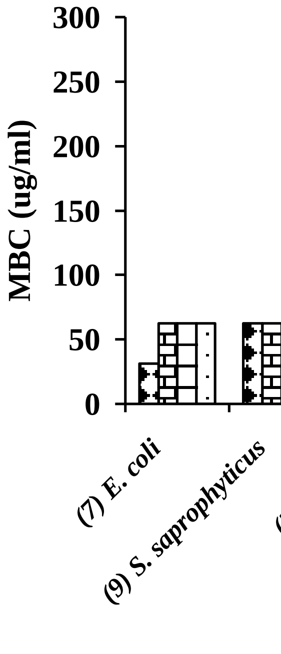
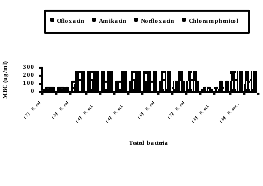


Fig. (9): Minimum inhibitory concentration (MICs) (µg/ml) of different antibiotics against tested bacteria.


MBC (ug/ml)
300
250
200
150
100
50
0
(7) E. coli
(9) S. saprophyticus



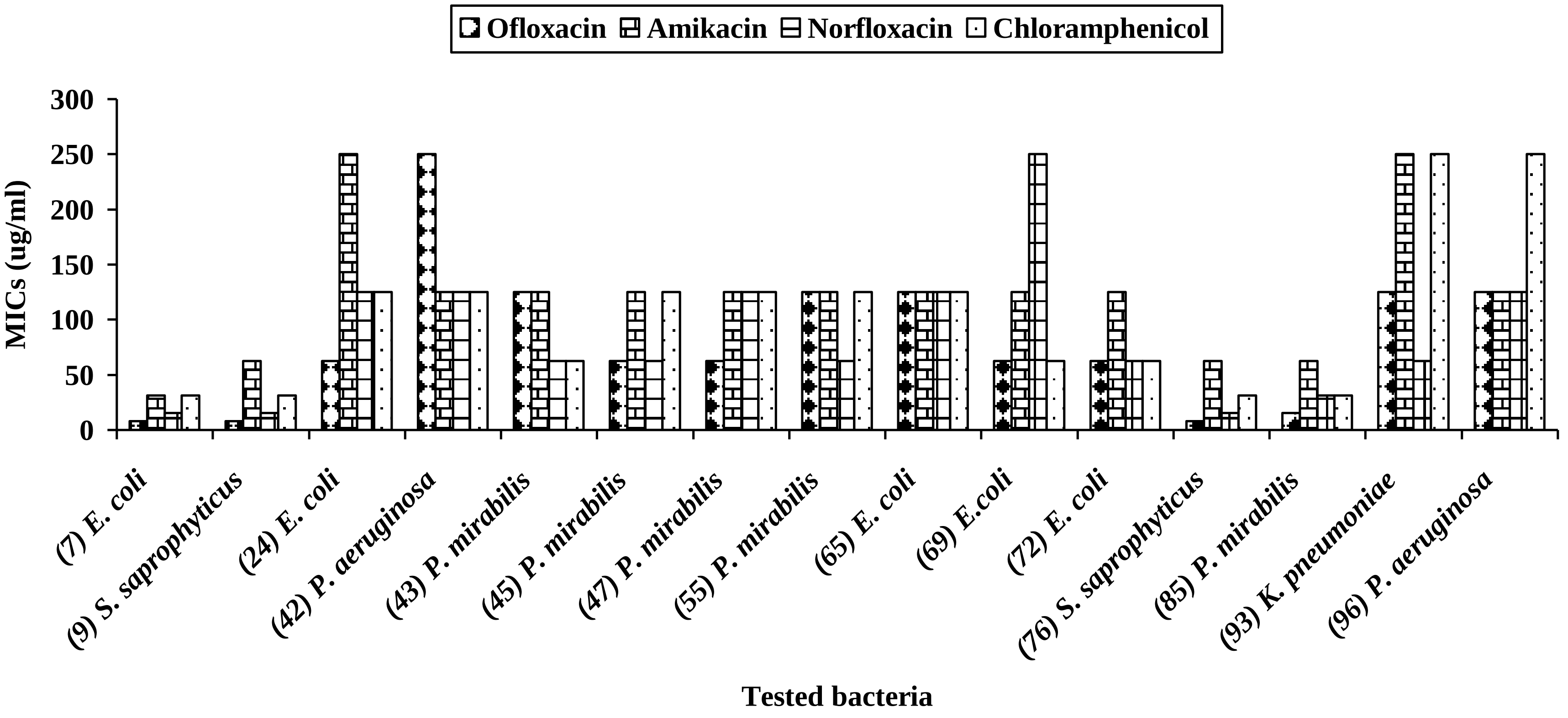


Fig. (9): Minimum inhibitory concentration (MICs) (µg/ml) of different antibiotics against tested bacteria

11- Antagonistic effect of different clinical medicinal plant extracts (cold & boiled water and alcohol extract) against selected bacterial isolates.

In this experiment, an attempt was made to test the antagonistic effect of different medicinal plant extracts (rosemary, orange peel, garlic, lemon grass, peppermint, spearmint, hibiscus, marjoram, thyme, tilia, clove, fennel, cinnamon, castor plant, ginger and chamomile), as cold water extract, boiled water extract and alcoholic extract against selected clinical bacterial isolates *E. coli* number 7, 24, 65 69 & 72, *P. aeruginosa* number 42 & 96, *K. pneumoniae* number 93, *Proteus mirabilis* number 43, 45, 47, 55 & 85 and *Staphylococcus saprophyticus* number 9 & 76, as illustrated in table (14, 15, 16 & 17) and represent graphically in fig. (11).

11. a- Antagonistic effect of cold water extracts.

The results given in table (14) and photos No. (3) showed that the cold water extracts of orange peel, spearmint, fennel and tilia have not any antagonistic effect against tested clinical bacterial isolates. On the other hand chamomile, marjoram, ginger and lemon grass extracts showed weak antagonistic effect on most bacterial isolates, but cold water extract of ginger give strong antagonistic activity against *P. mirabilis* number 47 (20 mm).

In generally castor plant, thyme, peppermint and rosemary showed moderate effect against growth of most clinical bacterial isolates which give less than 20 mm inhibition zone.

Garlic, hibisicus, cinnamon and clove cold water extract were given strong antagonistic effect against growth of most tested bacterial isolates, where in garlic extract give 20, 23, 25, 20, 28, 22, 30 and 25 mm inhibition zones against *S. saprohyticus* number 9, *E. coli* number 24, *P. mirabilis* number 47, *P. mirabilis* number 55, *E. coli* number 65, *E. coli* number 72*, S. saprohyticus* number 76 and *P. mirabilis* number 85 respectively. Hibisicus extract gave 25, 20, 20 and 20 mm against growth of *E. coli* number 7, *P. mirabilis* number 55, *S. saprohyticus* number 76 and *K. pneumoniae* number 93 respectively. Clove extract gave 22 mm against growth of *E. coli* number 24 and *P. aeruginosa* number 42 and 20 mm against *P. mirabilis* number 45, *P. mirabilis* number 55, *E. coli* 69 and *S. saprohyticus* number 76 respectively.

11. b- Antagonistic effect of boiling water extracts.

The results given in table (15) and photos No. (4) showed that the boiling water extracts of garlic, spearmint and tilia have not any antagonistic effect against tested clinical bacterial isolates. On the other hand chamomile, fennel, castor plant, thyme, marjoram, orange peel, ginger and lemon grass extracts showed weak antagonistic effect on most bacterial isolates.

In generally peppermint and cinnamon showed moderate effect against growth of most clinical bacterial isolates which give less than 20 mm inhibition zone.

Rosmary, hibisicus and clove boiling water extract were given strong antagonistic effect against growth of most tested bacterial isolates, where in Rosmary extract give 20 and 20 mm inhibition zones against *P. aeruginosa* number 42 and *Proteus mirabilis* number 45. Hibisicus extract gave 20 mm against growth of *Proteus mirabilis* number 55. Clove extract gave 20, 20, 20, 20, 22 and 20 mm against growth of *E. coli* number 7, *S. saprohyticus* number 9, *P. mirabilis* number 43, *P. mirabilis* number 55, *E. coli* number 72 and *K. pneumoniae* number 93 respectively.

11. c- Antagonistic effect of alcoholic extracts.

The results given in table (16) and photos No. (5) showed that the alcoholic extracts of spearmint and tilia have not any antagonistic effect against tested clinical bacterial isolates. On the other hand chamomile, fennel, cinnamon, orange peel and ginger extracts showed weak antagonistic effect on most bacterial isolates, but alcoholic extract of fennel give strong antagonistic activity against *P. mirabilis* number 45 (20 mm).

In generally lemon grass and thyme showed moderate effect against growth of most clinical bacterial isolates which give less than 20 mm inhibition zone.

Garlic, peppermint, marjoram, castor plant, hibisicus and clove alcoholic extract were given strong antagonistic effect against growth of most tested bacterial isolates, where in garlic extract give 25, 25, 20, 35 and 20 mm inhibition zones against *saprohyticus* number 9, *Proteus mirabilis* number 45, *Proteus mirabilis* number 55, *saprohyticus* number 76 and *P. aeruginosa* number 96 respectively. Peppermint extract gave 22 mm against growth of *E. coli* number 65. Marjoram extract gave 20, 22, 20, 25 and 20 mm against growth of *P. mirabilis* number 43, *P. mirabilis* number 45, *P. mirabilis* number 55, *E. coli* number 65 and *P. aeruginosa* number 96 respectively. Castor plant extract gave 20 and 20 mm inhibition zones against *P. mirabilis* number 47 and *E. coli* number 69. Hibisicus extract gave 20 mm inhibition zone against *E. coli* number 7. Clove extract gave 20 mm inhibition zones against *saprohyticus* number 9, *Proteus mirabilis* number 45 and *E. coli* number 65.

11. d- Statistical analysis.

Statistical analysis show in table 17 illustrated that the results incase orange peel, garlic, peppermint, cinnamon and fennel are high significant, while incase castor plant and marjoram are significant, but incase spearmint, hibisicus, rosemary, lemon grass, thyme, clove, chamomile, tilia and ginger are not significant.

Table (14): Antibacterial activities of different cold water medicinal plant extract.

Bacterial isolates	No.	Diameter of inhibition zones (mm)															
		Rosemary	Orange peel	Garlic	Lemon grass	Peppermint	Spearmint	Hibiscus	Marjoram	Thyme	Cinnamon	Castor plant	Clove	Fennel	Tilia	Ginger	Chamomile
		IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ
E. coli	7	**11**	**ND**	**ND**	**10**	**15**	**ND**	**25**	**16**	**8**	**9**	**ND**	**15**	**ND**	**ND**	**ND**	**ND**
S. saprophyticus	9	**11**	**ND**	**20**	**13**	**ND**	**ND**	**12**	**15**	**13**	**12**	**12**	**18**	**ND**	**ND**	**ND**	**ND**
E. coli	24	**13**	**ND**	**23**	**ND**	**11**	**ND**	**17**	**ND**	**9**	**9**	**11**	**22**	**ND**	**ND**	**ND**	**ND**
P. aeruginosa	42	**15**	**ND**	**ND**	**9**	**ND**	**ND**	**15**	**ND**	**10**	**9**	**12**	**22**	**ND**	**ND**	**ND**	**ND**
P. mirabilis	43	**15**	**ND**	**ND**	**9**	**12**	**ND**	**12**	**ND**	**12**	**ND**	**12**	**ND**	**ND**	**ND**	**ND**	**12**
P. mirabilis	45	**12**	**ND**	**15**	**ND**	**12**	**ND**	**15**	**ND**	**11**	**12**	**10**	**20**	**ND**	**ND**	**ND**	**ND**
P. mirabilis	47	**11**	**ND**	**25**	**ND**	**ND**	**ND**	**16**	**ND**	**ND**	**9**	**10**	**18**	**ND**	**ND**	**20**	**11**
P. mirabilis	55	**14**	**ND**	**20**	**10**	**12**	**ND**	**20**	**12**	**13**	**12**	**10**	**20**	**ND**	**ND**	**ND**	**ND**
E. coli	65	**ND**	**ND**	**28**	**9**	**ND**	**ND**	**13**	**11**	**11**	**ND**	**10**	**18**	**ND**	**ND**	**14**	**ND**
E. coli	69	**12**	**ND**	**19**	**12**	**11**	**ND**	**13**	**ND**	**10**	**10**	**10**	**20**	**ND**	**ND**	**ND**	**13**
E. coli	72	**14**	**ND**	**22**	**13**	**16**	**ND**	**12**	**10**	**9**	**8**	**ND**	**13**	**ND**	**ND**	**ND**	**11**
S. saprophyticus	76	**12**	**ND**	**30**	**9**	**ND**	**ND**	**20**	**ND**	**11**	**20**	**ND**	**20**	**ND**	**ND**	**ND**	**14**
P. mirabilis	85	**16**	**ND**	**25**	**ND**	**11**	**ND**	**15**	**ND**	**12**	**13**	**12**	**15**	**ND**	**ND**	**ND**	**10**
K. pneomoniae	93	**11**	**ND**	**15**	**13**	**12**	**ND**	**20**	**10**	**ND**	**ND**	**11**	**15**	**ND**	**ND**	**ND**	**ND**
P. aeruginosa	96	**12**	**ND**	**15**	**ND**	**15**	**ND**	**16**	**15**	**12**	**20**	**13**	**17**	**ND**	**ND**	**ND**	**ND**

IZ=Inhibition zone
ND=Not detected (0.0)

Table (15): Antibacterial activities of different boiling water medicinal plant extract.

Bacterial isolates	No.	Diameter of inhibition zones (mm)															
		Rosemary	Orange peel	Garlic	Lemon grass	Peppermint	Spearmint	Hibiscus	Marjoram	Thyme	Cinnamon	Castor plant	Clove	Fennel	Tilia	Ginger	Chamomile
		IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ
E. coli	7	**17**	**10**	**ND**	**11**	**14**	**ND**	**12**	**15**	**12**	**12**	**11**	**20**	**ND**	**ND**	**ND**	**ND**
S. saprophyticus	9	**17**	**ND**	**ND**	**10**	**14**	**ND**	**18**	**ND**	**ND**	**10**	**ND**	**20**	**ND**	**ND**	**ND**	**11**
E. coli	24	**15**	**11**	**ND**	**13**	**14**	**ND**	**17**	**15**	**12**	**11**	**ND**	**18**	**ND**	**ND**	**13**	**ND**
P. aeruginosa	42	**20**	**ND**	**ND**	**11**	**17**	**ND**	**15**	**17**	**13**	**12**	**12**	**17**	**ND**	**ND**	**ND**	**ND**
P. mirabilis	43	**18**	**ND**	**ND**	**10**	**14**	**ND**	**16**	**16**	**13**	**12**	**12**	**20**	**10**	**ND**	**ND**	**12**
P. mirabilis	45	**20**	**ND**	**ND**	**11**	**15**	**ND**	**15**	**16**	**15**	**11**	**ND**	**15**	**ND**	**ND**	**13**	**ND**
P. mirabilis	47	**16**	**ND**	**ND**	**11**	**15**	**ND**	**11**	**ND**	**ND**	**12**	**10**	**17**	**12**	**ND**	**10**	**ND**
P. mirabilis	55	**16**	**ND**	**ND**	**10**	**16**	**ND**	**20**	**17**	**15**	**12**	**ND**	**20**	**ND**	**ND**	**10**	**ND**
E. coli	65	**13**	**12**	**ND**	**12**	**10**	**ND**	**13**	**11**	**12**	**16**	**10**	**18**	**ND**	**ND**	**15**	**ND**
E. coli	69	**17**	**ND**	**ND**	**10**	**16**	**ND**	**14**	**ND**	**11**	**13**	**11**	**18**	**ND**	**ND**	**ND**	**12**
E. coli	72	**15**	**ND**	**ND**	**15**	**13**	**ND**	**12**	**15**	**14**	**17**	**ND**	**22**	**ND**	**ND**	**ND**	**11**
S. saprophyticus	76	**13**	**12**	**ND**	**ND**	**11**	**ND**	**15**	**ND**	**13**	**10**	**ND**	**12**	**ND**	**ND**	**ND**	**ND**
P. mirabilis	85	**15**	**ND**	**ND**	**12**	**17**	**ND**	**12**	**ND**	**16**	**10**	**13**	**17**	**ND**	**ND**	**ND**	**ND**
K. pneomoniae	93	**16**	**ND**	**ND**	**11**	**13**	**ND**	**15**	**12**	**ND**	**12**	**10**	**20**	**11**	**ND**	**ND**	**ND**
P. aeruginosa	96	**15**	**ND**	**ND**	**13**	**12**	**ND**	**15**	**16**	**11**	**12**	**12**	**16**	**ND**	**ND**	**ND**	**12**

IZ=Inhibition zone
ND=Not detected (0.0)

Table (16): Antibacterial activities of different alcoholic medicinal plant extract.

Bacterial isolates	No.	Diameter of inhibition zones (mm) and susceptibility of alcoholic plant extract															
		Rosemary	Orange peel	Garlic	Lemon grass	Peppermint	Spearmint	Hibiscus	Marjoram	Thyme	Cinnamon	Castor plant	Clove	Fennel	Tilia	Ginger	Chamomile
		IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ	IZ
E. coli	7	17	10	ND	12	16	ND	20	12	17	ND	15	18	13	ND	ND	18
S. saprophyticus	9	13	16	25	ND	16	ND	15	ND	8	15	15	20	11	ND	ND	ND
E. coli	24	20	12	ND	17	18	ND	13	17	15	ND	15	18	ND	ND	13	10
P. aeruginosa	42	20	14	ND	16	10	ND	12	11	18	ND	12	18	10	ND	ND	ND
P. mirabilis	43	20	10	ND	15	17	ND	17	20	10	ND	14	17	ND	ND	ND	10
P. mirabilis	45	18	15	25	17	13	ND	14	22	12	10	18	20	20	ND	13	10
P. mirabilis	47	16	10	12	16	18	ND	15	18	15	18	20	17	ND	ND	10	17
P. mirabilis	55	18	11	20	18	18	ND	16	20	12	ND	17	18	12	ND	10	ND
E. coli	65	18	14	11	16	22	ND	15	25	11	ND	19	20	14	ND	15	10
E. coli	69	15	ND	15	10	17	ND	15	18	11	12	20	13	12	ND	ND	12
E. coli	72	13	12	10	11	17	ND	16	11	11	9	12	15	10	ND	ND	10
S. saprophyticus	76	17	ND	35	12	17	ND	18	13	15	10	15	19	11	ND	12	13
P. mirabilis	85	17	ND	12	15	12	ND	17	12	15	15	18	17	10	ND	ND	10
K. pneomoniae	93	15	ND	ND	14	18	ND	16	10	16	11	15	16	12	ND	ND	17
P. aeruginosa	96	10	ND	20	11	17	ND	17	20	10	ND	17	10	13	ND	ND	ND

IZ=Inhibition zone
ND=Not detected (0.0)

Table (17): Sensitivity of bacterial isolates against cold water, boiled water and alcoholic plant extracts.

Bacterial isolates	No. of bacterial isolates against																							
	Rosemary extraction by						Orange peel extraction by						Garlic extraction by						Lemon grass extraction by					
	Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol	
	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
E. coli	1	4	0	5	0	5	5	0	2	3	1	4	1	4	5	0	2	3	1	4	0	5	0	5
Proteus mirabilis	0	5	0	5	0	5	5	0	5	0	1	4	1	4	5	0	1	4	3	2	0	5	0	5
K. pneumoniae	0	1	0	1	0	1	1	0	1	0	1	0	0	1	1	0	1	0	0	1	0	1	0	1
P. aeruginosa	0	2	0	2	0	2	2	0	2	0	1	1	1	1	2	0	1	1	1	1	0	2	0	2
S. saprophyticus	0	2	0	2	0	2	2	0	1	1	1	1	0	2	2	0	0	2	0	2	1	1	1	1
Total	1	14	0	15	0	15	15	0	11	4	5	10	3	12	15	0	5	10	5	10	1	14	1	14
X^2	2.05						15.76						22.06						5.41					
P	0.35 NS						0.001 HS						0.001 HS						0.06 NS					

Continuous table (17).

Bacterial isolates	No. of bacterial isolates against																							
	Peppermint extraction by						Spearmint extraction by						Hibiscus extraction by						Marjoram extraction by					
	Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol	
	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
E. coli	1	4	0	5	0	5	5	0	5	0	5	0	0	5	0	5	0	5	2	3	1	4	0	5
Proteus mirabilis	1	4	0	5	0	5	5	0	5	0	5	0	0	5	0	5	0	5	4	1	2	3	0	5
K. pneumoniae	0	1	0	1	0	1	1	0	1	0	1	0	0	1	0	1	0	1	0	1	0	1	0	1
P. aeruginosa	1	1	0	2	0	2	2	0	2	0	2	0	0	2	0	2	0	2	1	1	0	2	0	2
S. saprophyticus	2	0	0	2	0	2	2	0	2	0	2	0	0	2	0	2	0	2	1	1	2	0	1	1
Total	5	10	0	15	0	15	15	0	15	0	15	0	0	15	0	15	0	15	8	7	5	10	1	14
X^2	11.25						0.0						0.0						7.67					
P	0.003 HS						1.0 NS						1.0 NS						0.02 S					

Continuous table (17).

Bacterial isolates	No. of bacterial isolates against																							
	Thyme extraction by						Cinnamon extraction by						Castor plant extraction by						Clove extraction by					
	Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol	
	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
E. coli	0	5	0	5	0	5	1	4	0	5	3	2	2	3	2	3	0	5	0	5	0	5	0	5
Proteus mirabilis	1	4	1	4	0	5	1	4	0	5	2	3	0	5	2	3	0	5	1	4	0	5	0	5
K. pneumoniae	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1
P. aeruginosa	0	2	0	2	0	2	0	2	0	2	2	0	0	2	0	2	0	2	0	2	0	2	0	2
S. saprophyticus	0	2	1	1	0	2	0	2	0	2	0	2	1	1	2	0	0	2	0	2	0	2	0	2
Total	2	13	3	12	0	15	3	12	0	15	7	8	3	12	6	9	0	15	1	14	0	15	0	15
X^2	3.15						9.51						7.5						2.05					
P	0.2 NS						0.008 HS						0.023 S						0.35 NS					

Continuous table (17).

Bacterial isolates	No. of bacterial isolates against																							
	Fennel extraction by						Tilia extraction by						Ginger extraction by						Chamomile extraction by					
	Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol		Cold water		Boiled water		Alcohol	
	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
E. coli	5	0	5	0	1	4	5	0	5	0	5	0	4	1	3	2	5	0	3	2	2	3	0	5
Proteus mirabilis	5	0	3	2	2	3	5	0	5	0	5	0	4	1	2	3	5	0	2	3	4	1	1	4
K. pneumoniae	1	0	0	1	0	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	0	1
P. aeruginosa	2	0	2	0	0	2	2	0	2	0	2	0	2	0	2	0	2	0	2	0	1	1	2	0
S. saprophyticus	2	0	2	0	0	2	2	0	2	0	2	0	2	0	2	0	1	1	1	1	1	1	1	1
Total	15	0	12	3	3	12	15	0	15	0	15	0	13	2	10	5	14	1	9	6	9	6	4	11
X^2	23.4						0.0						3.95						4.45					
P	0.001 HS						1.0 NS						0.13 NS						0.1 NS					

ND = Not detected (not effective against bacteria) (0.0).
D = Detected (effective against bacteria).

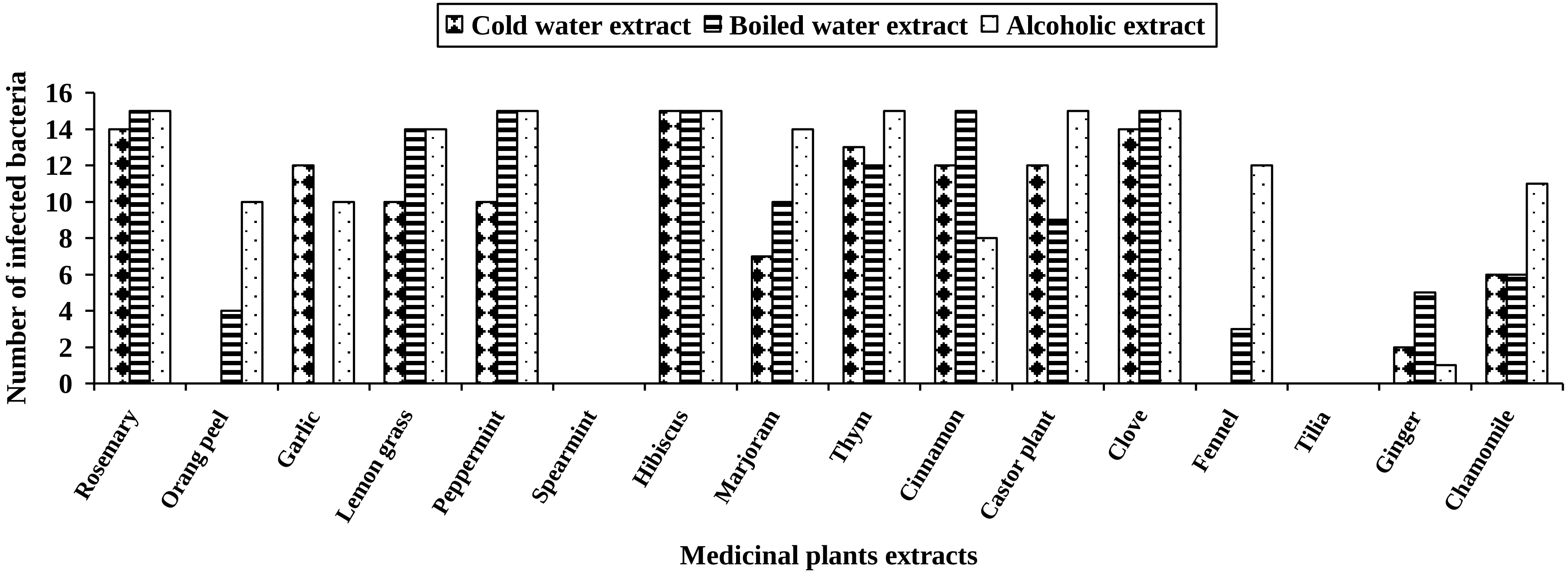
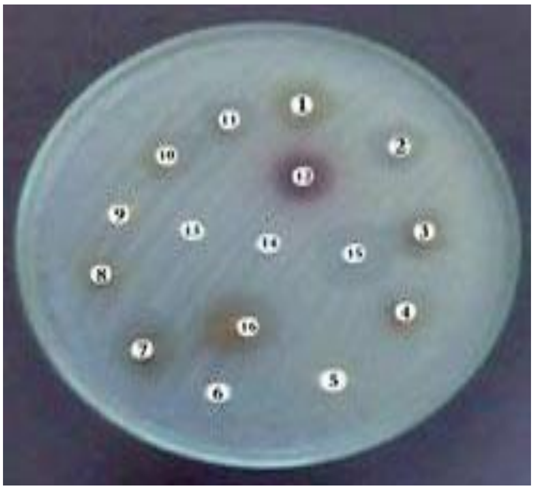
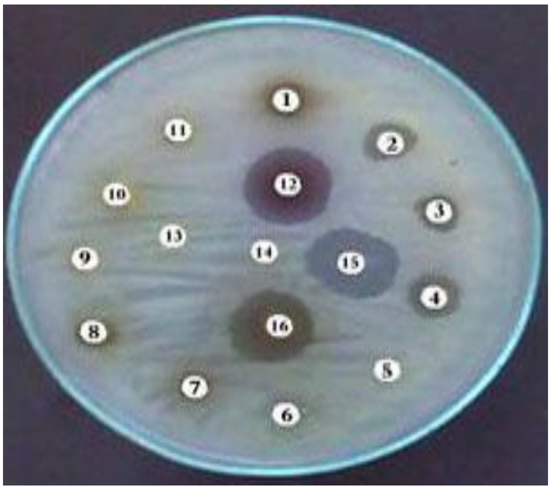


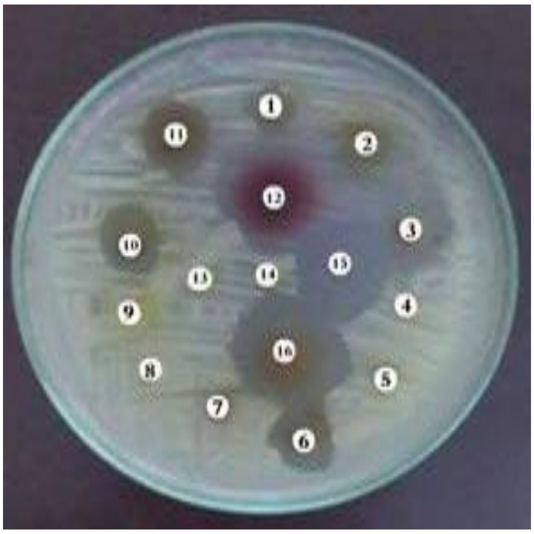
Fig. (11): Sensitivity of bacterial isolates against cold water, boiled water and alcoholic plant extracts.



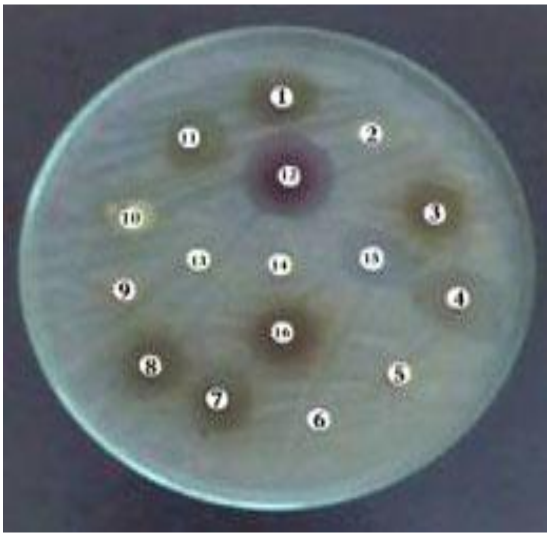
E. coli **number 69**



Staphylococcus saprophyticus **number 76**



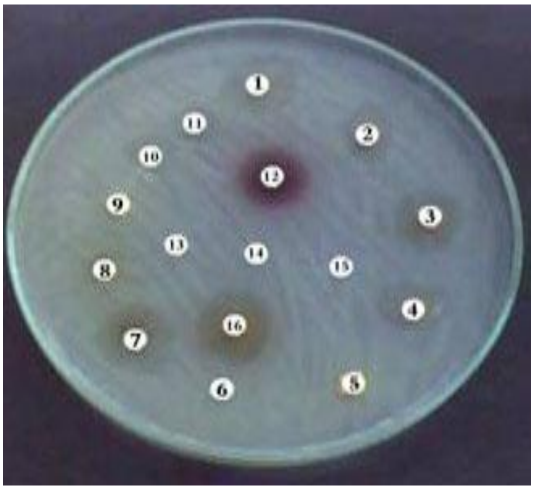
Proteus mirabilis **number 85**



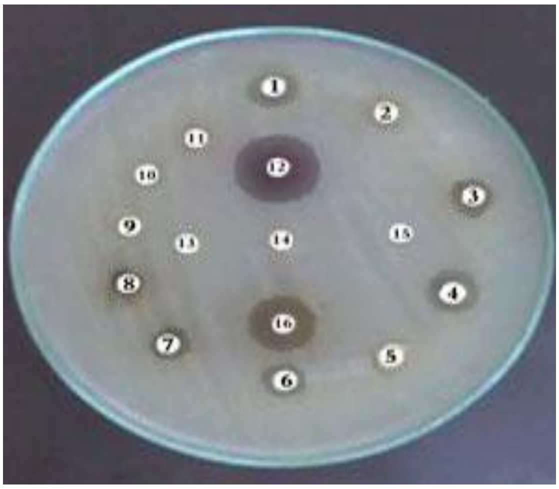
Pseudomonas aeruginosa **number 96**

Photos No. (3): Effect of cold water medicinal plants extracts against bacterial isolates by disc diffusion method.

1- Rosemary, 2- Chamomile, 3- Thyme, 4- Cinnamon, 5- Ginger, 6- Orange peel, 7- Peppermint, 8- Marjoram, 9- Fennel, 10- Lemon grass, 11- Castor plant, 12- Hibiscus, 13- Tilia, 14- Spearmint, 15- Garlic, 16- Clove.

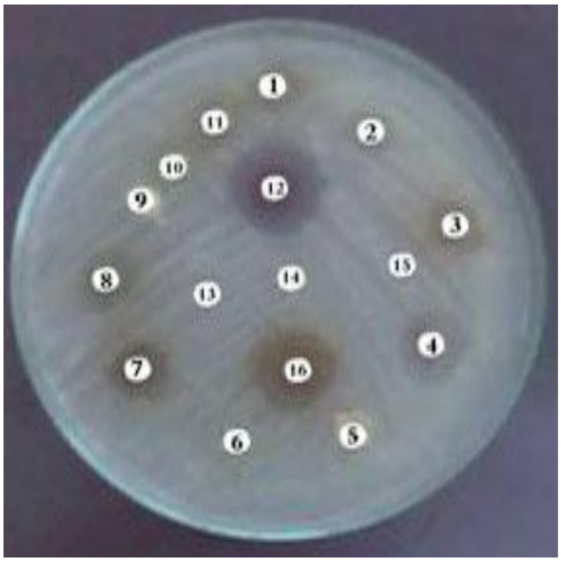


E. coli **number 69**



Staphylococcus saprophyticus **number 76**

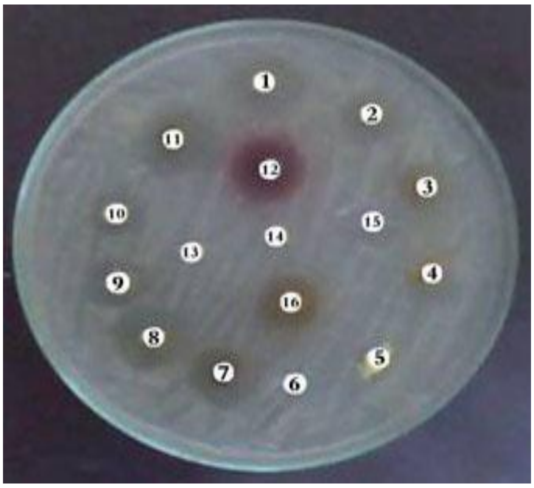
Proteus mirabilis **number 85**



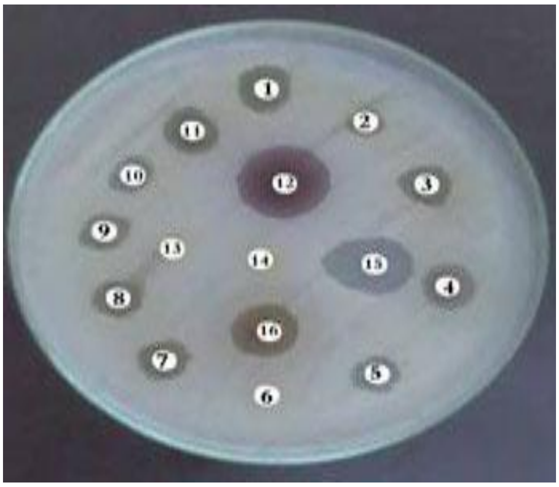
Pseudomonas aeruginosa **number 96**

Photos No. (4): Effect of boiled water medicinal plants extracts against bacterial isolates by disc diffusion method.

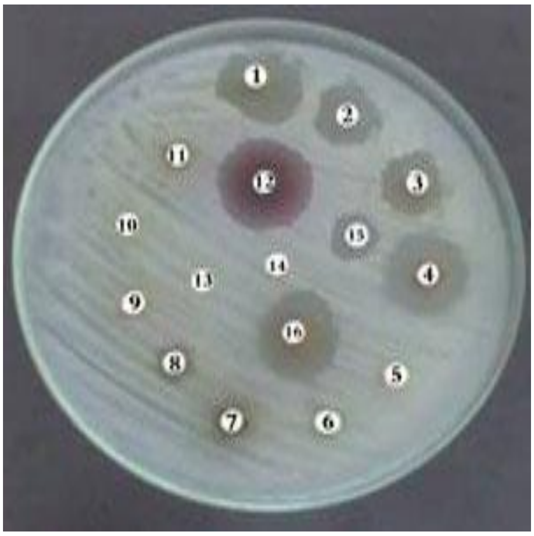
1- Rosemary, 2- Chamomile, 3- Thyme, 4- Cinnamon, 5- Ginger, 6- Orange peel, 7- Peppermint, 8- Marjoram, 9- Fennel, 10- Lemon grass, 11- Castor plant, 12- Hibiscus, 13- Tilia, 14- Spearmint, 15- Garlic, 16- Clove.



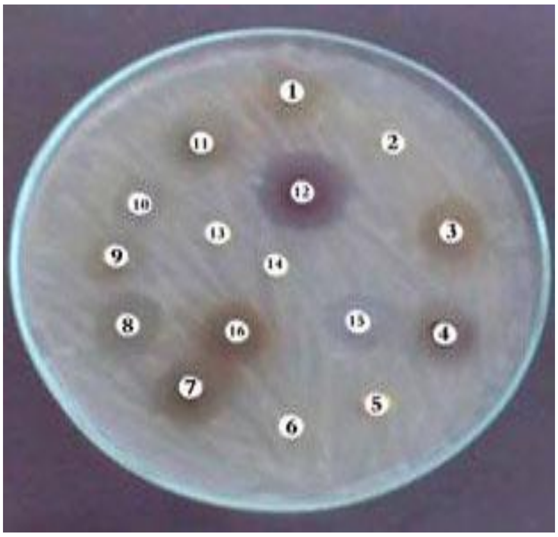
E. coli **number 69**



Staphylococcus saprophyticus **number 76**



Proteus mirabilis **number 85**



Pseudomonas aeruginosa **number 96**

Photos No. (5): Effect of alcoholic medicinal plants extracts against bacterial isolates by disc diffusion method.

1- Rosemary, 2- Chamomile, 3- Thyme, 4- Cinnamon, 5- Ginger, 6- Orange peel, 7- Peppermint, 8- Marjoram, 9- Fennel, 10- Lemon grass, 11- Castor plant, 12- Hibiscus, 13- Tilia, 14- Spearmint, 15- Garlic, 16- Clove.

12- The effect of combination between different medicinal plants extracts and MICs of some antibiotics on clinical bacterial isolates.

The medicinal plants extracts that used in this experiment were rosemary, garlic, peppermint, hibiscus, clove and castor plant, which combined with the MICs of ofloxacin and amikacin for each clinical bacterial isolates to study the effect of combination between them and their action against bacterial isolates will increase than effect of singly used or not, as illustrated in photos No. (6, 7).

Extraction of rosemary, peppermint and castor plant by alcohol, while extraction of garlic and hibiscus by cold water and extraction of clove by boiling water, were used which recorded the best extraction gave the highest antagonistic activities against clinical bacterial isolates.

The result of combination effect were recorded in table (18), which indicated that the combination between MIC of ofloxacin antibiotics with plant extract rosemary clearly synergistic action against *E. coli* number 7, 24, 65 & 69, *P. aeruginosa* number 42 & 96, *K. pneumoniae* number 93 and *P. mirabilis* number 45 more than singly used of antibiotic ofloxacin or rosemary extract. On the other hand the antagonistic effect clearly illustrated with combination between rosemary extract and ofloxacin against *S. saprophyticus* number 9 & 76, *E. coli* number 72 and *P. mirabilis* number 43, 47, 55 & 85, which recorded inhibition action against these bacterial isolates less than singly used antibiotics.

Synergistic effect of combination between ofloxacin and hibisicus, peppermint and clove were recorded against *P. mirabilis* number 43 which obtained 20, 25 & 23 mm respectively. Antagonistic effect were clearly obtained at peppermint, garlic and castor oil against *S. saprophyticus* number 9, *E. coli* number 24 & 69 and *P. mirabilis* number 85, which did not record any inhibition zones.

The result of combination effect were recorded in table (19), which indicated that the combination between MIC of amikacin antibiotics with plant extract rosemary clearly synergistic action against *E. coli* number 65, 69 & 72, *P. aeruginosa* number 42 & 96, *K. pneumoniae* number 93, *P. mirabilis* number 43, 45, 4.7 & 85 and *S. saprophyticus* number 9 & 76 more than singly used of antibiotic amikacin or rosemary extract. On the other hand the antagonistic effect clearly illustrated with combination between rosemary extract and amikacin against *E. coli* number 7 & 24 and *P. mirabilis* number 55, which recorded inhibition action against these bacterial isolates less than singly used antibiotics.

Synergistic effect of combination between amikacin and hibisicus, peppermint and clove were recorded against *S. saprophyticus* number 76 which obtained 27, 20 & 25 mm respectively. Antagonistic effect were clearly obtained at garlic and castor oil against *S. saprophyticus* number 9, *E. coli* number 65, 69 & 72, *P. mirabilis* number 85 and *K. pneumoniae* number 93, which did not record any inhibition zones.

Table (18): Combination effect between different medicinal plant extracts and MICs of ofloxacin against bacterial isolates.

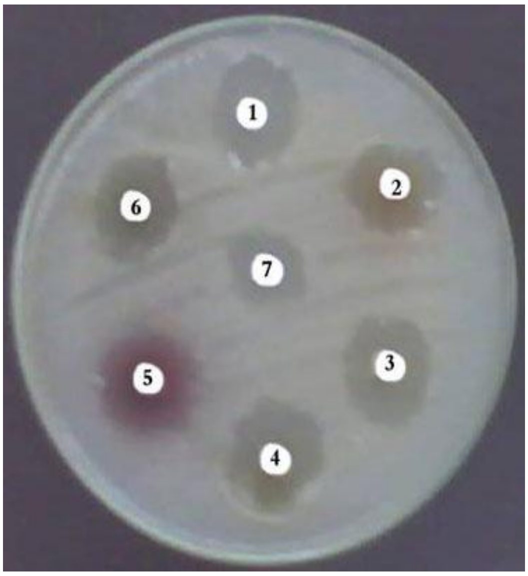
Bacterial isolates	No.	Ofloxacin MICs		Diameter of inhibition zones (mm) of medicinal plant extracts					
				Rosemary	Garlic	Clove	Castor plant	Peppermint	Hibiscus
		µg/ml	IZ	IZ	IZ	IZ	IZ	IZ	IZ
E. coli	7	7.8125	12	**14**	**12**	**13**	**12**	**12**	**12**
S. saprophyticus	9	7.8125	12	**10**	**11**	**15**	**ND**	**ND**	**15**
E. coli	24	62.5	12	**15**	**ND**	**18**	**13**	**14**	**15**
P. aeruginosa	42	250	15	**17**	**15**	**18**	**11**	**15**	**17**
P. mirabilis	43	125	15	**15**	**12**	**23**	**ND**	**25**	**20**
P. mirabilis	45	62.5	12	**22**	**15**	**20**	**ND**	**17**	**20**
P. mirabilis	47	62.5	12	**ND**	**12**	**18**	**12**	**13**	**16**
P. mirabilis	55	125	15	**15**	**12**	**20**	**11**	**20**	**22**
E. coli	65	125	14	**17**	**12**	**15**	**14**	**13**	**18**
E. coli	69	62.5	11	**15**	**ND**	**19**	**ND**	**15**	**14**
E. coli	72	62.5	15	**ND**	**16**	**17**	**11**	**10**	**12**
S. saprophyticus	76	7.8125	15	**15**	**18**	**18**	**17**	**11**	**23**
P. mirabilis	85	15.625	15	**10**	**12**	**20**	**ND**	**10**	**20**
K. pneomoniae	93	125	13	**23**	**10**	**20**	**23**	**21**	**20**
P. aeruginosa	96	62.5	12	**14**	**12**	**18**	**10**	**15**	**14**
$\bar{X}$ **±SD**			13.3 ±1.5	**15.5 ±3.7**	**13.0 ±2.3**	**18.1 ±2.5**	**13.4 ±3.9**	**15.1 ±4.3**	**17.2 ±3.5**
t				**2.08**	**0.45**	**6.37**	**0.05**	**1.44**	**3.88**
P				**0.04 S**	**0.65 NS**	**0.001 HS**	**0.9 NS**	**0.15 NS**	**0.001 HS**

IZ = Inhibition zone
ND = Not detected (0.0)

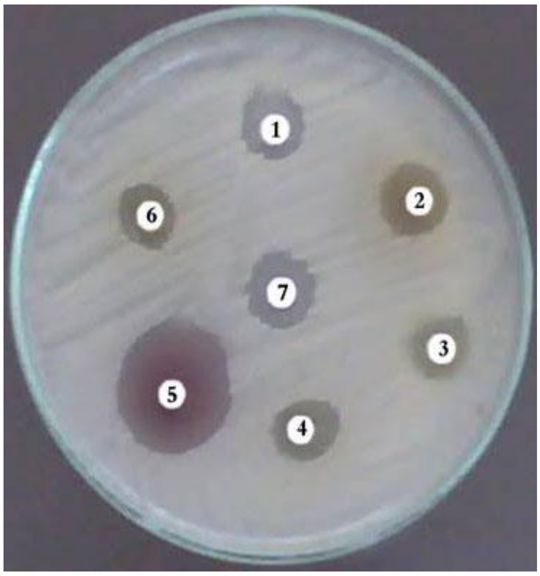
Table (19): Combination effect between different medicinal plant extracts and MICs of amikacin against bacterial isolates.

Bacterial isolates	No.	Amikacin MICs		Diameter of inhibition zones (mm) of medicinal plant extracts					
				Rosemary	Garlic	Clove	Castor plant	Peppermint	Hibiscus
		µg/ml	IZ	IZ	IZ	IZ	IZ	IZ	IZ
E. coli	7	31.25	12	12	16	15	12	12	14
S. saprophyticus	9	62.5	12	15	10	16	12	14	13
E. coli	24	250	12	11	11	20	13	14	13
P. aeruginosa	42	125	10	14	12	15	11	14	18
P. mirabilis	43	125	12	15	12	19	19	14	13
P. mirabilis	45	125	10	12	14	15	13	12	15
P. mirabilis	47	125	11	12	11	18	13	14	22
P. mirabilis	55	125	13	13	10	18	22	15	12
E. coli	65	125	13	16	ND	15	17	15	15
E. coli	69	125	10	13	ND	17	10	12	20
E. coli	72	125	12	15	ND	15	ND	10	14
S. saprophyticus	76	62.5	13	22	26	25	15	20	27
P. mirabilis	85	62.5	10	14	10	18	ND	12	17
K. pneomoniae	93	250	13	15	ND	16	15	12	20
P. aeruginosa	96	125	15	18	12	19	13	12	21
$\bar{X}$ ±SD			11.9 ±1.5	14.5 ±2.8	13.1 ±4.6	17.4 ±2.7	14.2 ±3.4	13.5 ±2.3	16.9 ±4.2
t				3.2	0.96	6.94	2.46	2.27	4.32
P				0.003 HS	0.65 NS	0.001 HS	0.019 S	0.02 S	0.001 HS

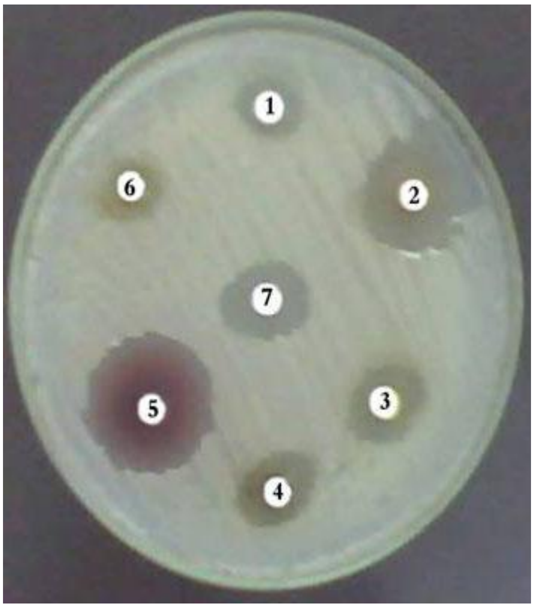
IZ = Inhibition zone
ND = Not detected (0.0)



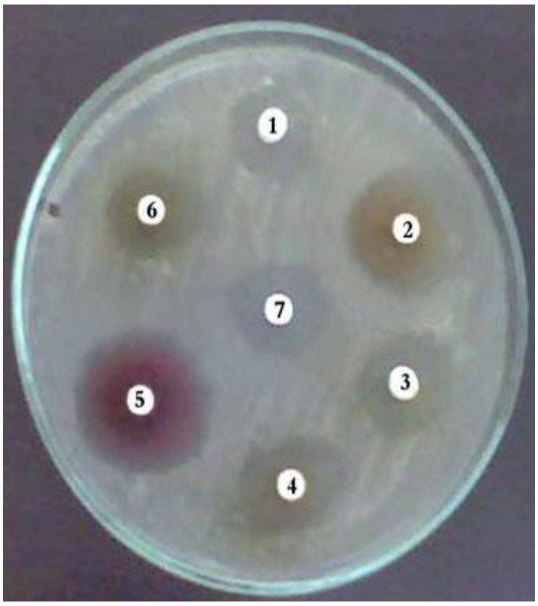
E. coli **number 7**



Staphylococcus saprophyticus **number 76**



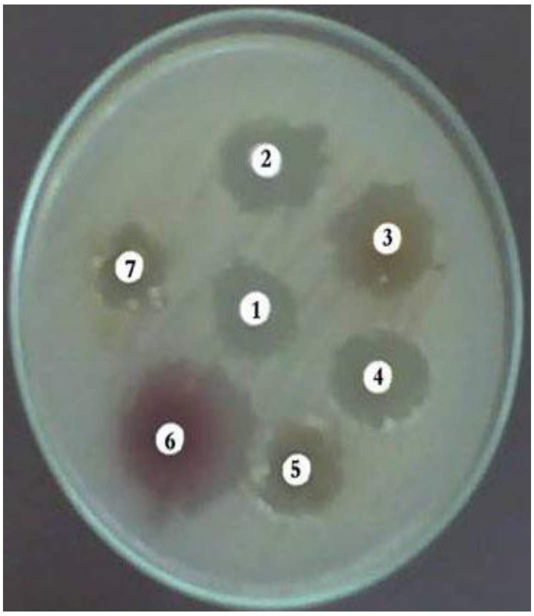
Proteus mirabilis **number 85**



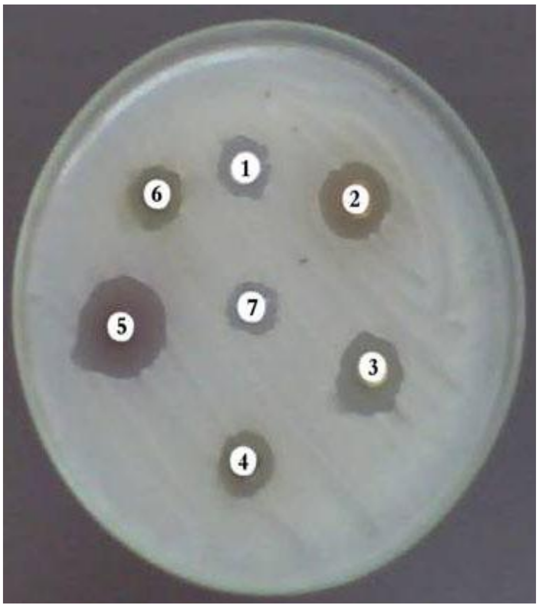
Pseudomonas aeruginosa **number 96**

Photos No. (6): Combination between medicinal plants extracts and ofloxacin antibiotic against bacterial isolates by disc diffusion method.

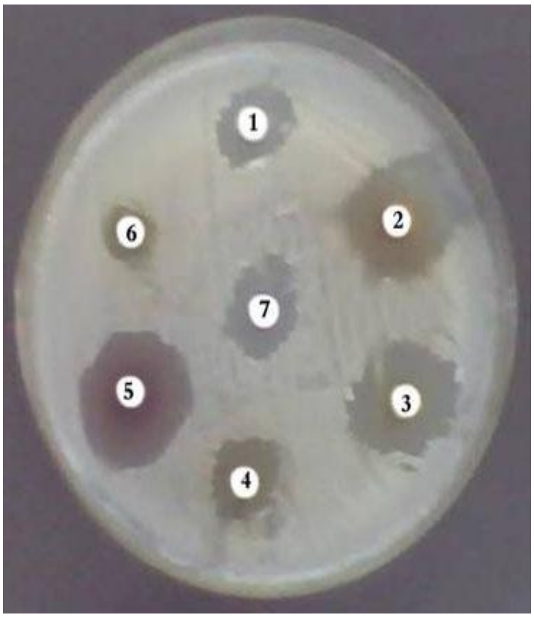
1- Garlic, 2- Clove, 3- Rosemary, 4- Peppermint, 5- Hibiscus, 6- Castor plant, 7- MIC of ofloxacin.



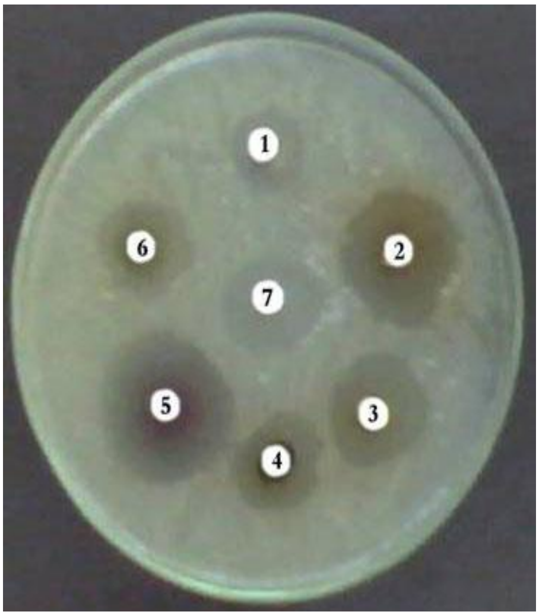
E. coli **number 7**



Staphylococcus saprophyticus **number 76**



Proteus mirabilis **number 85**



Pseudomonas aeruginosa **number 96**

Photos No. (7): Combination between medicinal plants extracts and amikacin antibiotic against bacterial isolates by disc diffusion method.

1- Garlic, 2- Clove, 3- Rosemary, 4- Peppermint, 5- Hibiscus, 6- Castor plant, 7- MIC of amikacin.

13- The effect of *Hibiscus sabdariffa* extract after neutalization for its high acidity by 1 ml of NaOH (1 N).

Hibiscus sabdariffa extract has a strong acidity where pH equal to 2.48, while other plants extracts recorded weak acidic pH ranged 5.00 - 6.13.

It was thought that the high acidity of *Hibiscus sabdariffa* extract was the reason that causes inhibition for bacterial growth, so the neutralization of hibisicus cold water extract was performed.

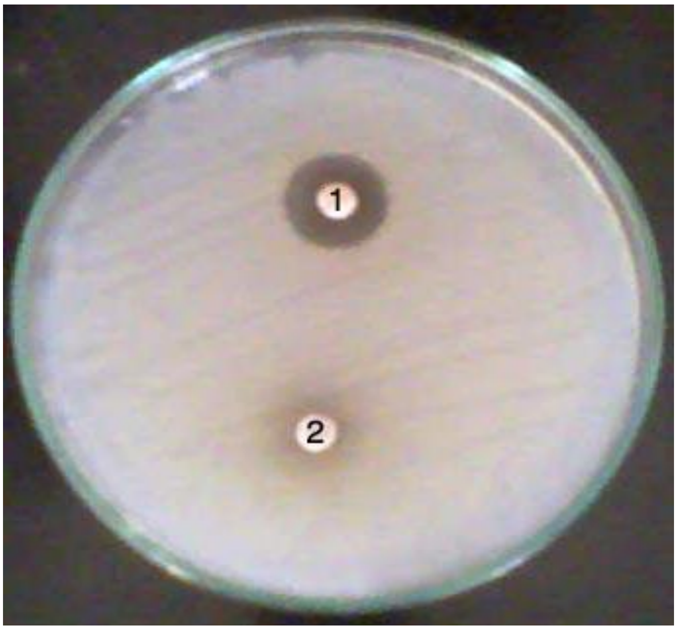
The results in table (20) and photos No. (8) confirmed that neutralized pH of *Hibiscus sabdariffa* extract haven't any antibacterial effectivity on tested isolated, while *Hibiscus sabdariffa* extract have antibacterial effectivity on tested isolated.

So, the acidity of *Hibiscus sabdariffa* is the reason for inhibition bacterial growth, as the results obtained in table (20), which indicated that the antibacterial activities of neutralized extract of hibisicus not recorded any inhibition zone against all tested clinical bacterial isolates if compared with neutral extracts which recorded inhibition zones between 12-20 mm.

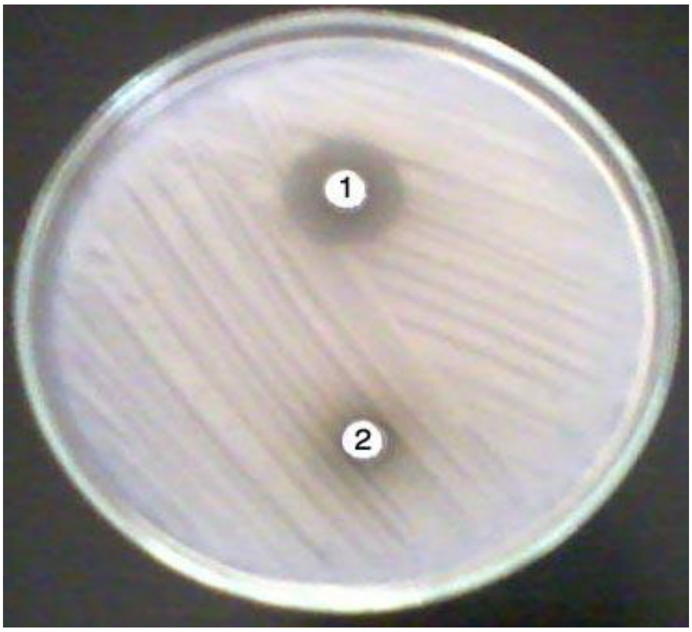
Table (20): Diameter of inhibition zone (mm) of *Hibiscus sabdariffa* extract at normal pH and neutralized pH.

Bacterial isolates	**No.**	**Diameter of inhibition zones (mm) of *Hibiscus sabdariffa* extract**	
		Normal pH	**Neutralized pH**
		IZ	**IZ**
E. coli	7	15	ND
S. saprophyticus	9	18	ND
E. coli	24	17	ND
P. aeruginosa	42	15	ND
P. mirabilis	43	16	ND
P. mirabilis	45	15	ND
P. mirabilis	47	14	ND
P. mirabilis	55	20	ND
E. coli	65	17	ND
E. coli	69	14	ND
E. coli	72	12	ND
S. saprophyticus	76	15	ND
P. mirabilis	85	14	ND
K. pneomoniae	93	18	ND
P. aeruginosa	96	15	ND

IZ = Inhibition zone
ND = Not detect (0.0)



Proteus mirabilis **number 85**



Klebsiella pneumoniae **number 93**

Photos No. (8): Effect of *Hibisicus sabdariffa* extract against bacterial isolates before and after neutralization by disc diffusion method.

1- Hibisicus normal extract, 2- Hibisicus neutralized extract.

14- Protein analysis for clinical bacterial isolates under stress of clove extract and ofloxacin antibiotic.

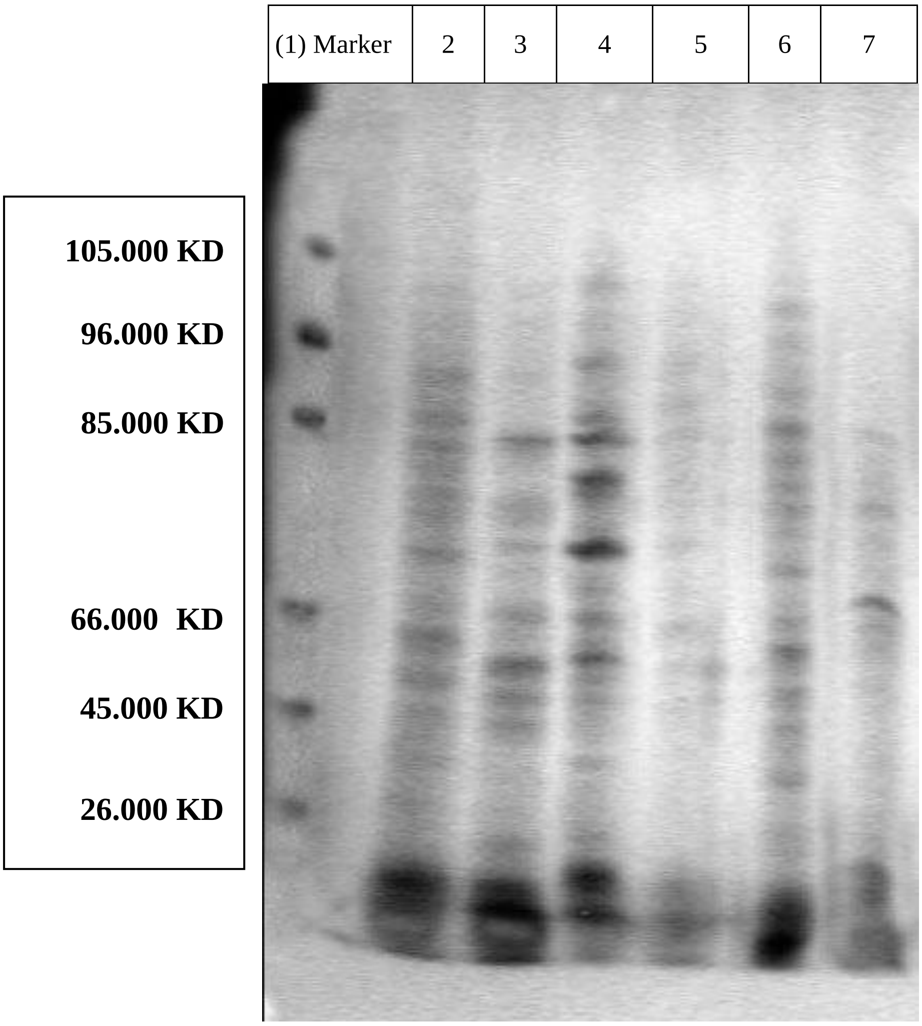
The experimental clinical bacterial isolates were grown on nutrient agar without any stress at normal condition as control samples, and also grown on nutrient agar under stress of combination between boiling water extract of clove and the concentration of antibiotics ofloxacin (sub MIC concentration) for each bacterial isolates as treated samples.

The tested bacterial isolates were *P. aeruginosa* number 42, *P. mirabilis* number 55 and *K. pneumoniae* number 93 and the antibiotic ofloxacin concentration were 125, 62.5 & 62.5 µg/ml respectively. The clove extract is concentrated.

The results in table (21) and photos No. (9) indicated that there is difference in protein bands between bacteria before and after treatment, where incase *P. aeruginosa* number 42 the molecular weight for each protein bands were (21.921, 25.765, 29.644, 41.405, 75.545, 85.594 & 107.811) KD were disappear after treatment, on other hand the molecular weight for new protein bands (23.241, 32.438, 35.677, 44.08 & 97.613) KD were appear after treatment. Incase *P. mirabilis* number 55 the molecular weight for each protein bands were (20.683, 23.241, 25.765, 28.218, 32.438, 34.06, 35.677, 38.233, 46.419, 63.901, 85.594 & 107.811) KD were disappear after treatment, on other hand the molecular weight for new protein bands (19.364 & 57.365) KD were appear after treatment. And in *K. pneumoniae* number 93 the molecular weight for each protein bands were (24.321, 28.218, 32.438, 35.677, 41.405, 44.0852.362, 57.365, 63.901, 75.545, 85.594, 97.613 & 107.811) KD were disappear after treatment, on other hand the molecular weight for new protein bands (23.241, 25.765 & 46.419) KD were recorded in *K. pneumoniae*.

Table (21): Protein analysis for clinical bacterial isolates under stress of clove extract and ofloxacin antibiotic.

Band No.	MW (KD)	Marker	*P. aeruginosa* number 42		*P. mirabilis* number 55		*K. peumoniae* number 93	
			Control	Treated	Control	Treated	Control	Treated
		Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
1	107.811		1	0	1	0	1	0
2	97.613		0	1	0	0	1	0
3	85.594		1	0	1	0	1	0
4	75.545		1	0	1	1	1	0
5	70.785		1	1	1	1	1	1
6	63.901		0	0	1	0	1	0
7	60.323		1	1	0	0	1	1
8	57.365		0	0	0	1	1	0
9	52.362		1	1	1	1	1	0
10	46.419		0	0	1	0	0	1
11	44.08		0	1	0	0	1	0
12	41.405		1	0	1	1	1	0
13	38.233		1	1	1	0	0	0
14	35.677		0	1	1	0	1	0
15	34.06		0	0	1	0	0	0
16	32.438		0	1	1	0	1	0
17	29.644		1	0	0	0	0	0
18	28.218		0	0	1	0	1	0
19	25.765		1	0	1	0	0	1
20	24.321		0	0	0	0	1	0
21	23.241		0	1	1	0	0	1
22	21.921		1	0	0	0	0	0
23	20.683		1	1	1	0	1	1
24	19.364		0	0	0	1	0	0
25	18.525		0	0	1	1	1	1



Photos No. (9): Protein analysis for clinical bacterial isolates before and after stress.

1- Marker.

2- *Pseudomonas aeruginosa* number 42 before stress (control).

3- *Pseudomonas aeruginosa* number 42 after stress.

4- *Proteus mirabilis* number 55 before stress (control).

5- *Proteus mirabilis* number 55 after stress.

6- *Klebsiella pneumoniae* number 93 before stress (control).

7- *Klebsiella pneumoniae* number 93 after stress.

*Stress = Bacterial cultivation with clove extract combined with ofloxacin (sub MIC) antibiotics.

15- Purification and identification of the antibacterial extracted compound.

The separation of the active antibacterial constituents of the *Syzygium aromaticum* boiling water extract was carried out using thin layer chromatography (TLC). Each RF was eluted in ethanol 95% and filteration to test the antibacterial activities against tested bacteria.

RF No. 10 zone gave the antibacterial activities with all bacterial isolated from patients with urinary tract infection. While other rest RF showed no activities against tested bacterial isolates. The antibacterial activity of *Syzygium aromaticum* extract loaded on TLC was test against tested bacterial isolates and the results illustrated in Photos No. (10).

The IR and ^{1}HNMR spectrum for purified antimicrobial substances were conducted at the Microanalytical center of Cairo University, Egypt.

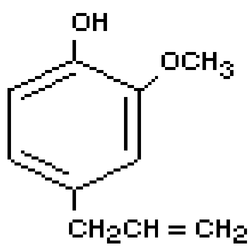
a- ^{1}HNMR spectrum

^{1}HNMR of compound showed signals at 3.8(d, 2H, CH_2), 5(t, 2H, $=CH_2$), 6(m, 1H, -CH=), 6.5-6.8(m, 3H, Ar H$^{'s}$) and at 8.6(S, 1H, OH); as illustrated in figure (12).

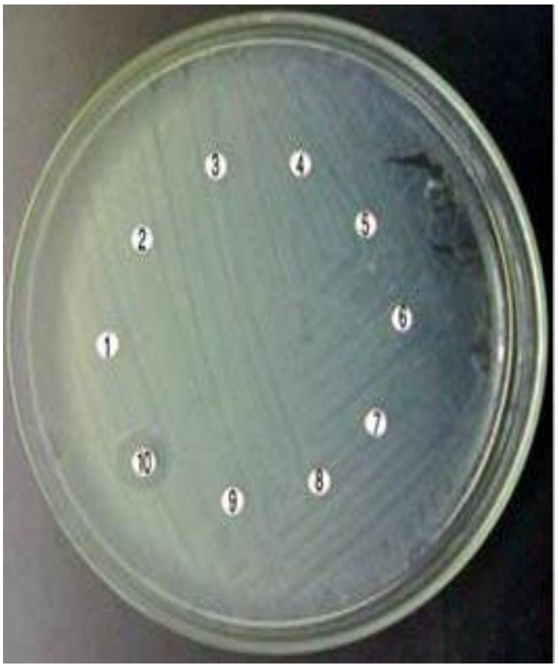
b- IR spectrum

IR of compound showed broad band at 3500 cm^{-1} corresponding to –OH group and band at 1518 cm^{-1} corresponding to C=C, in addition to O-C band absorption at 1099 cm^{-1}; as illustrated in figure (13).

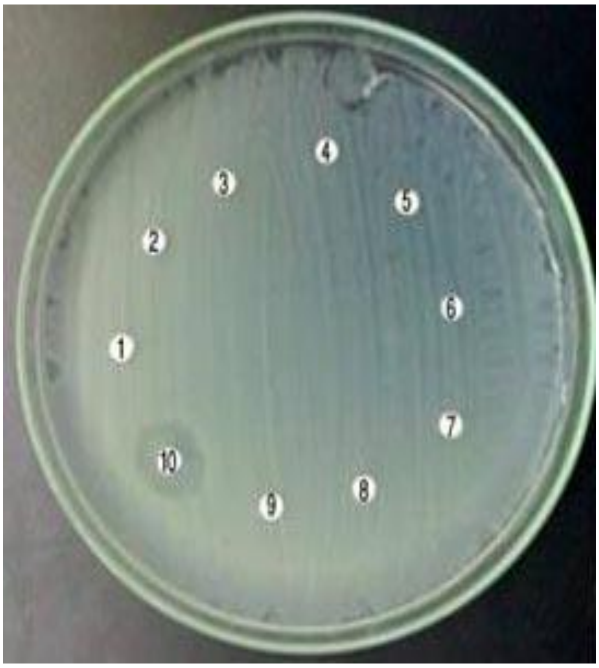
All data corresponding to Eugenol structure.



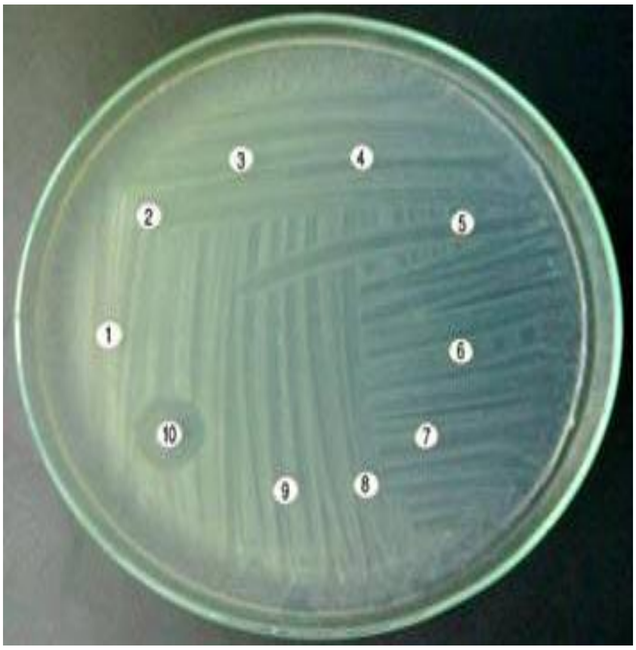
Eugenol



Proteus mirabilis **number 55**



***Staphylococcus saprophyticus* number 76**



***Klebsiella pneumoniae* number 93**

Photos No. (10): Antibacterial substances of different RF of clove extract against bacterial isolates by disc diffusion method.

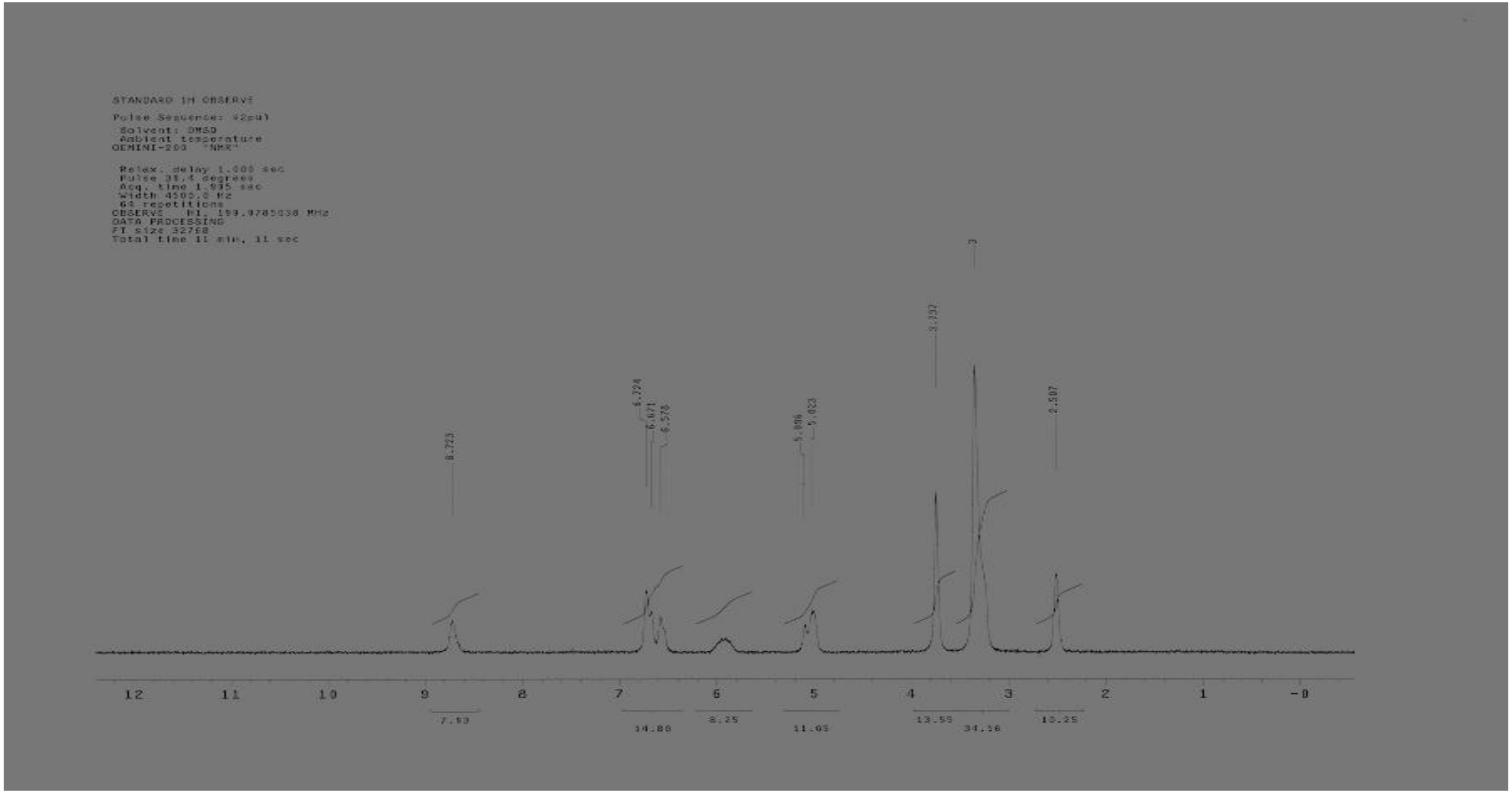
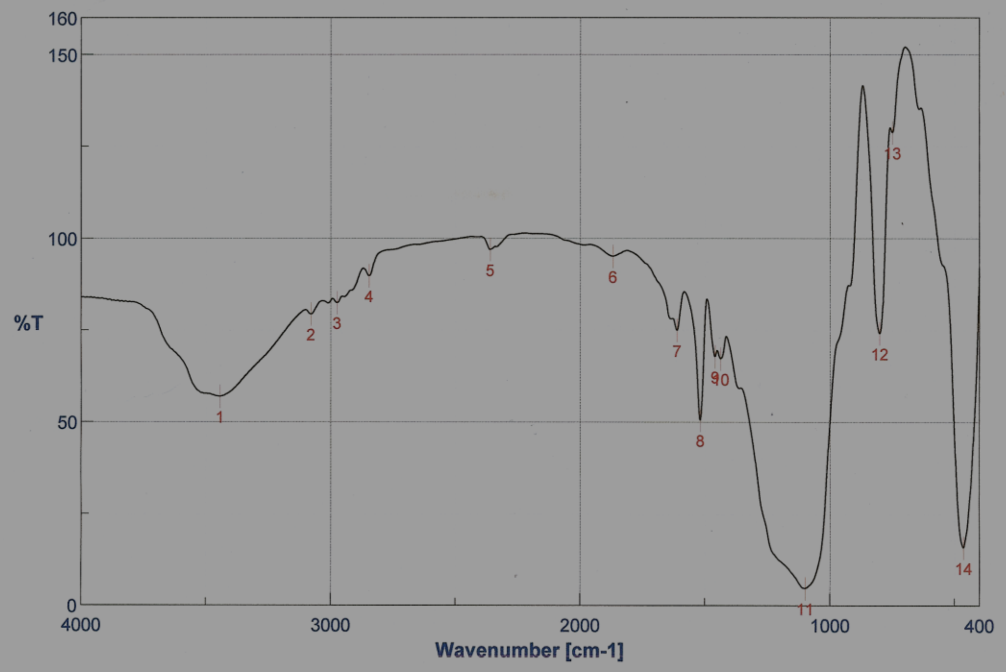


Fig (12): ^{1}HNMR spectrum of the antimicrobial substances.



Accumulation	16
Resolution	4 cm-1
Zero Filling	ON
Apodization	Cosine
Gain	Auto (4)
Scanning Speed	Auto (2 mm/sec)
Date/Time	25/03/2009 1:28ā
Update	25/03/2009 1:28ā
Operator	
File Name	Memory#31
Sample Name	
Comment	

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3444.24	56.954	2	3079.76	79.4028	3	2974.66	82.507
4	2846.42	89.8499	5	2361.41	96.9838	6	1869.65	95.2051
7	1612.2	75.0088	8	1518.67	50.6053	9	1459.85	67.86
10	1438.64	67.2862	11	1099.23	4.80964	12	801.278	74.1859
13	750.174	128.856	14	464.761	15.9619			

Fig. (13): IR spectrum of the antimicrobial substances.