RESULTS

1- Collection of urine samples from males and females.

The present study was conducted on 130 urine samples taken from patients who were examinated for UTIs in Zagazig University hospitals (in patients) or attending Zagazig University clinics (out patients), Urology Department. These samples have been collected from different ages of males and females ranged 9-84 years of males and 4-87 years of females. 66 patients of these cases were females , 50 were males and 14 urine samples were collected from healthy (not urinary tract infection) as control. These samples were collected from June 2008 to October 2008.

The diagnosis of UTIs as positive urine samples was based on the presence of $\geq 10^5$ CFU of microorganisms per ml in urine culture. The obtained results in Table (3) illustrated that 116 urine samples were positive for urinary tract infection , and 14 samples were negative(urinary tract infection not present). These samples collected from 49 outpatient and 67 inpatient.

The results illustrated in Table (4) showed that the highest number of infected urine male's samples was in the age from 41-52 years with 38% positive in urine males samples and 33.3 % positive in females urine samples.

The obtained results in Figure (1) illustrated that the positive collected samples of females was 66 cases more than the male cases which recorded 50 positive cases.

Table (3):Total bacterial count isolated from different collected urine samples

Urine samples No.	Age (years)	Gender	Inpatient& Outpatient	Total bacterial count (CFU/ml)
1	9	F	Inpatient	2.0×10^{6}
2	9	F	Inpatient	3.6×10^{6}
3	48	M	Inpatient	2.5×10^{6}
4	25	F	Outpatient	4.0×10^{6}
5	55	M	Outpatient	2.0×10^{5}
6	55	M	Outpatient	1.5×10^{5}
7	50	M	Outpatient	1.0×10^{6}
8	50	M	Inpatient	1.0×10^{5}
9	50	F	Outpatient	1.0×10^{6}
10	68	F	Inpatient	7.0×10^{4}
11	45	F	Inpatient	1.8×10^{6}
12	49	F	Inpatient	1.7×10^{6}
13	49	F	Outpatient	2.8×10^{6}
14	43	F	Inpatient	1.7×10^{6}
15	55	F	Inpatient	2.0×10^{6}
16	55	F	Inpatient	1.6×10^{6}
17	23	F	Outpatient	1.1×10^{6}
18	23	F	Outpatient	3.6×10^{6}
19	9	F	Outpatient	2.9×10^{6}
20	50	F	Outpatient	2.7×10^{6}
21	37	F	Inpatient	1.4×10^{5}
22	43	F	Inpatient	1.2×10^{5}
23	63	M	Inpatient	8.0×10^{4}

Continous Table (3)

Urine samples No.	**Age years)**	**Gender**	**Inpatient& Outpatient**	**Total bacterial count (CFU/ml)**
24	60	F	Inpatient	$1.0x10^5$
25	60	F	Inpatient	$3.0x10^6$
26	67	M	Inpatient	$2.7x10^6$
27	62	M	Inpatient	$1.1x10^6$
28	51	M	Inpatient	$1.0x10^6$
29	44	F	Inpatient	$3.5x10^6$
30	25	F	Outpatient	$3.0x10^6$
31	45	F	Outpatient	$2.9x10^6$
32	35	F	Outpatient	$7.0x10^5$
33	45	F	Inpatient	$1.0x10^5$
34	76	F	Inpatient	$4.0x10^6$
35	58	F	Inpatient	$2.7x10^6$
36	45	F	Outpatient	$6.0x10^5$
37	45	F	Inpatient	$9.0x10^5$
38	45	F	Inpatient	$9.0x10^4$
39	6	F	Inpatient	$2.5x10^6$
40	6	F	Outpatient	$7.0x10^4$
41	36	F	Inpatient	$3.6x10^6$
42	36	F	Outpatient	$8.0x10^4$
43	35	F	Outpatient	$1.08x10^6$
44	35	F	Inpatient	$1.2x10^5$
45	4	F	Inpatient	$2.4x10^6$
46	25	F	Outpatient	$7.0x10^5$

Continous Table (3)

Urine samples No.	**Age (years)**	**Gender**	**Inpatient& Outpatient**	**Total bacterial count (CFU/ml)**
47	45	M	Outpatient	$1.8x10^5$
48	35	M	Outpatient	$2.0x10^6$
49	50	F	Inpatient	$3.6x10^6$
50	50	F	Inpatient	$1.7x10^5$
51	60	F	Inpatient	$1.5x10^6$
52	50	M	Inpatient	$8.0x10^5$
53	50	M	Inpatient	$4.0x10^6$
54	60	M	Inpatient	$3.0x10^6$
55	55	M	Inpatient	$2.2x10^6$
56	40	M	Outpatient	$4.0x10^6$
57	26	F	Inpatient	$2.8x10^6$
58	26	F	Inpatient	$2.0x10^5$
59	27	F	Outpatient	$3.2x10^5$
60	27	F	Inpatient	$4.0x10^5$
61	30	F	Inpatient	$2.4x10^6$
62	40	F	Inpatient	$1.2x10^6$
63	45	M	Outpatient	$4.8x10^6$
64	50	M	Outpatient	$1.2x10^5$
65	13	M	Outpatient	$3.2x10^6$
66	35	F	Inpatient	$3.5x10^5$
67	40	F	Inpatient	$1.8x10^6$
68	45	F	Inpatient	$1.0x10^5$
69	60	M	Inpatient	$1.8x10^6$
70	40	M	Outpatient	$2.2x10^6$

Continous Table (3)

Urine samples No.	Age (years)	Gender	Inpatient& Outpatient	Total bacterial count (CFU/ml)
71	55	M	Inpatient	$1.4x10^6$
72	75	M	Inpatient	$5.0x10^5$
73	45	M	Outpatient	$2.4x10^6$
74	45	M	Outpatient	$1.0x10^5$
75	50	M	Inpatient	$2.6x10^6$
76	45	M	Inpatient	$2.0x10^6$
77	40	M	Inpatient	$2.4x10^6$
78	47	F	Inpatient	$2.0x10^5$
79	9	M	Inpatient	$1.4x10^5$
80	15	M	Outpatient	$2.1x10^6$
81	38	M	Outpatient	$1.0x10^5$
82	57	M	Outpatient	$2.6x10^6$
83	13	M	Outpatient	$2.5x10^6$
84	60	M	Outpatient	$1.2x10^6$
85	30	M	Outpatient	$8.0x10^4$
86	28	M	Inpatient	$2.5x10^6$
87	65	M	Inpatient	$3.0x10^6$
88	36	F	Inpatient	$7.0x10^5$
89	36	F	Inpatient	$1.4x10^6$
90	61	F	Outpatient	$2.5x10^6$
91	52	F	Inpatient	$3.6x10^6$
92	47	M	Inpatient	$2.8x10^6$
93	50	F	Outpatient	$8.0x10^4$
94	44	F	Outpatient	$2.0x10^6$

Continous Table (3)

Urine samples No.	Age (years)	Gender	Inpatient& Outpatient	Total bacterial count (CFU/ml)
95	7	F	Outpatient	$2.2x10^6$
96	87	F	Inpatient	$3.2x10^6$
97	40	F	Inpatient	$1.4x10^6$
98	52	M	Inpatient	$2.0x10^6$
99	60	M	Inpatient	$1.0x10^5$
100	84	M	Inpatient	$2.7x10^6$
101	70	M	Inpatient	$6.0x10^5$
102	18	F	Outpatient	$1.6x10^6$
103	33	F	Inpatient	$1.8x10^5$
104	42	M	Outpatient	$3.2x10^6$
105	70	M	Inpatient	$3.6x10^6$
106	45	M	Outpatient	$2.8x10^6$
107	48	M	Inpatient	$4.0x10^5$
108	25	F	Outpatient	$4.4x10^6$
109	32	F	Inpatient	$4.0x10^6$
110	44	F	Outpatient	$1.6x10^6$
111	44	F	Outpatient	$1.5x10^5$
112	49	F	Outpatient	$2.0x10^6$
113	54	M	Outpatient	$1.2x10^5$
114	50	M	Outpatient	$1.0x10^5$
115	76	M	Outpatient	$1.8x10^5$
116	40	M	Outpatient	$2.0x10^6$

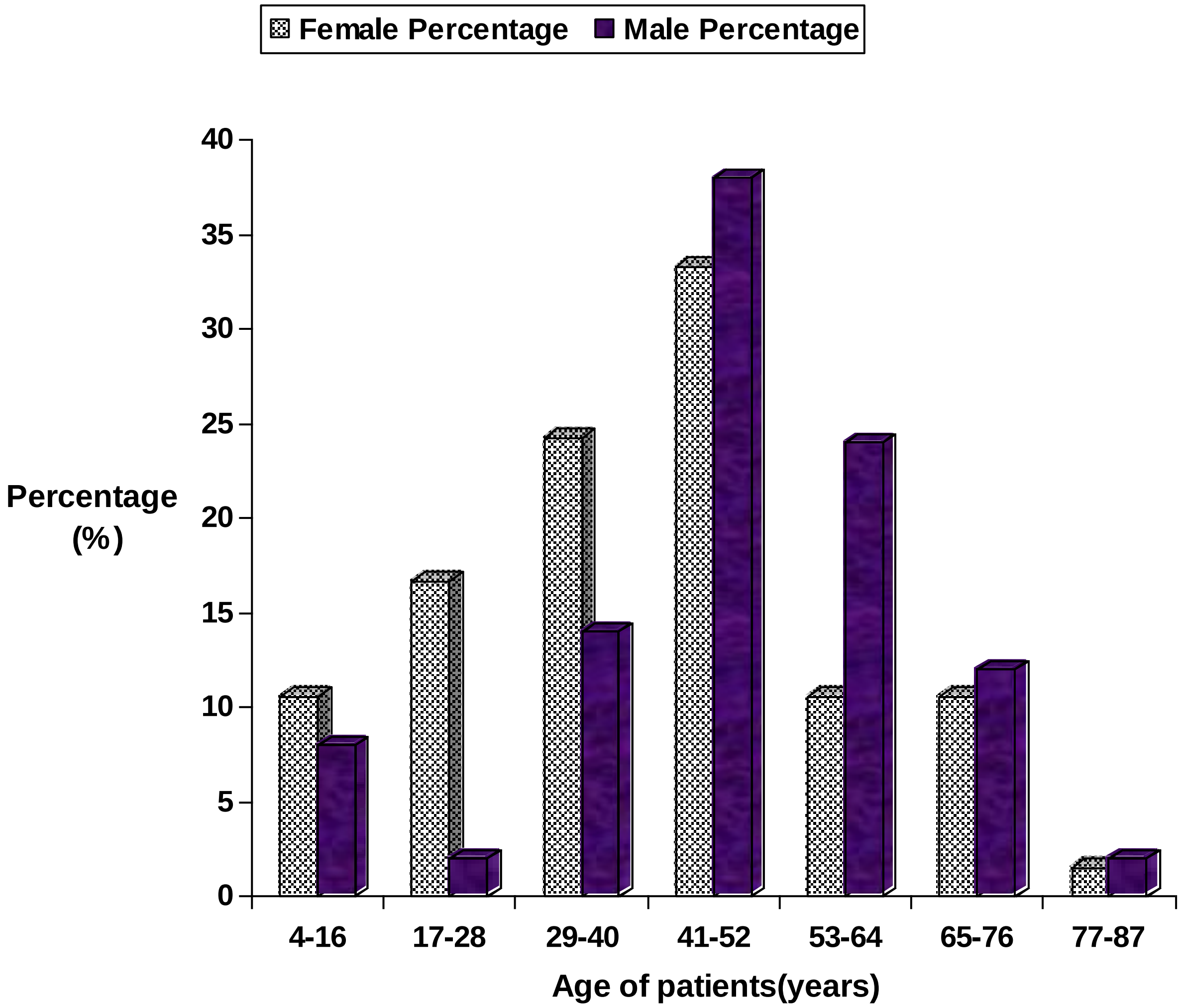
M=Male.

F=Female

Table (4): Percentage of positive urine samples collected from different ages of males and females patient.

Age (years)	Number of females positive samples	Percentage (%)	Number of males positive samples	Percentage (%)
4-16	7	10.6	4	8
17-28	11	16.7	1	2
29-40	16	24.3	7	14
41-52	22	33.3	19	38
53-64	7	10.6	12	24
65-76	2	3.0	6	12
77-87	1	1.5	1	2
4-87	66	100	50	100

$$\text{Percentage (\%)} = \frac{\text{Number of positive samples}}{\text{Total no. of samples (males or females)}} \times 100$$



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

2- Community –acquired and hospital – acquired UTIs

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

The obtained result in Table (5) and Figure (2) clearly demonstrated the highest percentage of hospital –acquired infection obtained was 57.8 while the percentage of community –acquired infection was 42.2 .

The percentage of females was 61.2 in hospital –acquired infection which more than percentage of males 38.8 . Also in the community –acquired infection , the percentage of females (51)were more than the percentage of males (49).

Table (5):Percentage of community –acquired and hospital- acquired UTIs in males and females:

UTIS	No. of patients	Percentage %	Males	Percentage %	Females	Percentage %
Community –acquired UTIs*	49	42.2	24	49	25	51
Hospital –acquired UTIs**	67	57.8	26	38.8	41	61.2
Total acquired UTIs	116	100	50	43.1	66	56.9
Total healthy individual (control)	14					

* Community –acquired infection = Outpatients

** Hospital – acquired infection =Inpatients

UTIs= Urinary tract infections.

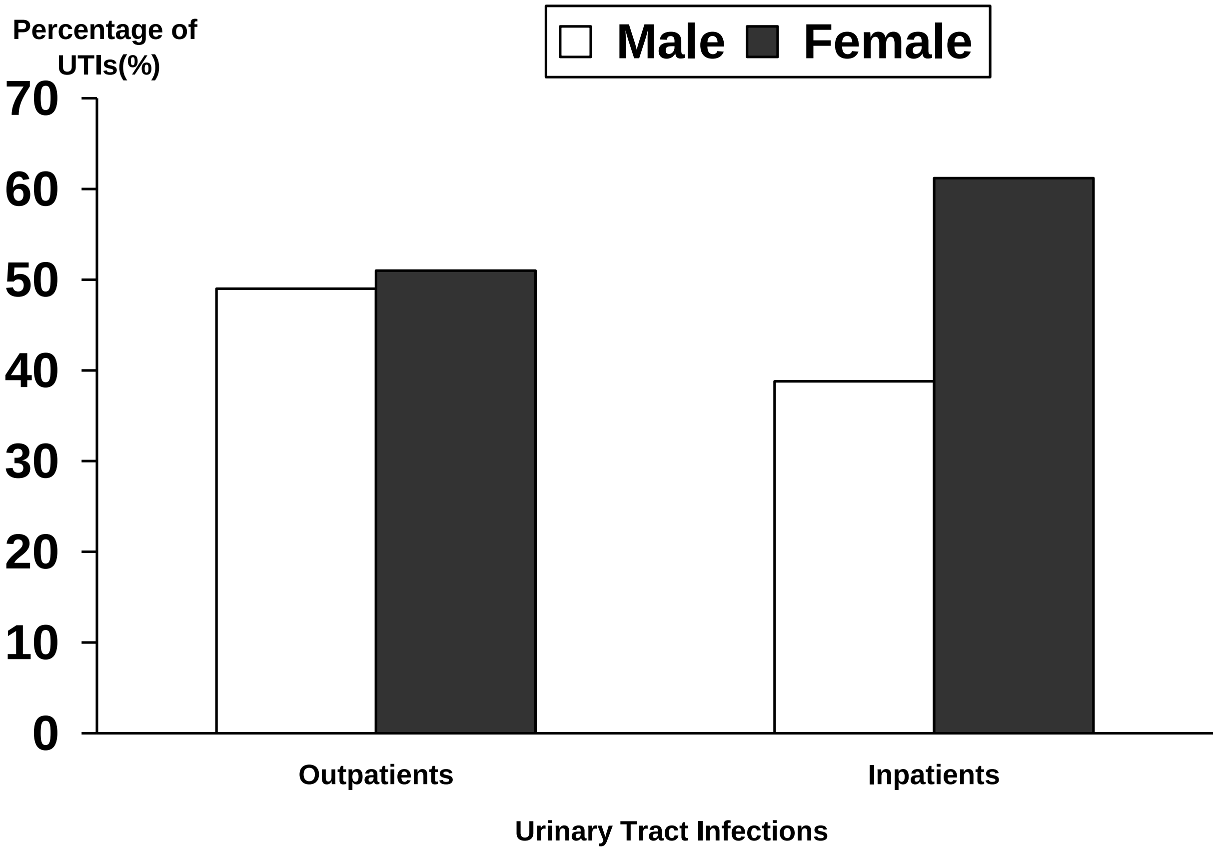


Fig (2):Percentage of community –acquired and hospital- acquired UTIs in males and females.

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3.1-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 Tables (6).

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, 72 isolates were Gram- negative bacilli, while 25 isolates were Gram- positive cocci and 19 isolates were fungi.

I-Gram –negative bacilli (72 isolates).

1-The first group of Gram- negative bacilli isolates includes 51 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 (TSI) test, (A/A/g+/H_2S-).

Gram staining and microscobic 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 51 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 17 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 staining and microscobic 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 17 isolates of the second group of Gram-negative bacilli were identified as strains belonging to *Klebsiella pneumonia.*

3-The third group of Gram- negative bacilli isolates: include 4 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 produce 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 microscobic 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 in case of oxidation only , lactose is negative , sucrose and mannitol are negative and maltose is positive.

According to the above characteristics the 4 isolates of the third group of Gram-negative bacilli were identified as strains belonging to *Pseudomonas aeruginosa* .

II-Gram- positive cocci (25 isolates)

Gram positive cocci isolates : include 25 isolates

Colonial morphology: Circular colonies , arranged in irregular grape like clusters.

Cultural properties: Facultative anaerobic, produce golden yellow colonies on nutrient agar and produce beta (complete) hemolysis on blood agar.

Gram staining and microscobic examination: Gram –positive cocci, non -capsulated and non spore-forming.

Biochemical characteristics: potassium hydroxide test negative, produce catalase enzyme, non motile, coagulase positive, can ferment mannitol with production of acid only.

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

According to the above characteristics the 25 isolates of this group of Gram-positive cocci were identified as strains belonging to *Staphylococcus aureus.*

3.2-Identification of isolated fungi: include 19 isolates.

Colonial morphology: Small , creamy, smooth, moist, raised and with yeast odour on sabouraud dextrose agar media.

Gram staining and microscobic examination: Gram positive budding yeast cells form pseudo or true hyphae.

Germ tube test: Positive.

According to the above characteristics the 19 isolates of this group of were identified as strains belonging to *Candida albicans*.

Table (6):Biochemical reaction of *E. coli, Klebsiella pneumonia , Pseudomonas aeruginosae and Staphylococcus aureus.*

Biochemical test	Bacterial isolates			
	E.coli	***Klebsiella pneumonia***	***Pseudomonas aeruginosae***	***Staphylococcus aureus***
Gram stain	-ve	-ve	-ve	+ve
KOH test	+ve	+ve	+ve	-ve
Catalase test	+ve	+ve	+ve	+ve
Oxidase test	-ve	-ve	+ve	-ve
Lactose	+ve	+ve	-ve	+ve
Glucose	+ve	+ve	+ve in O_2	+ve
Maltose	+ve	+ve	-ve	+ve
Sucrose	+ve	+ve	-ve	+ve
Mannitol	+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	Non -motile
Coagulase test				+ve
Novobiocin antibiotic				Sensitive

4- The distribution of pathogenic isolates from positive urine samples

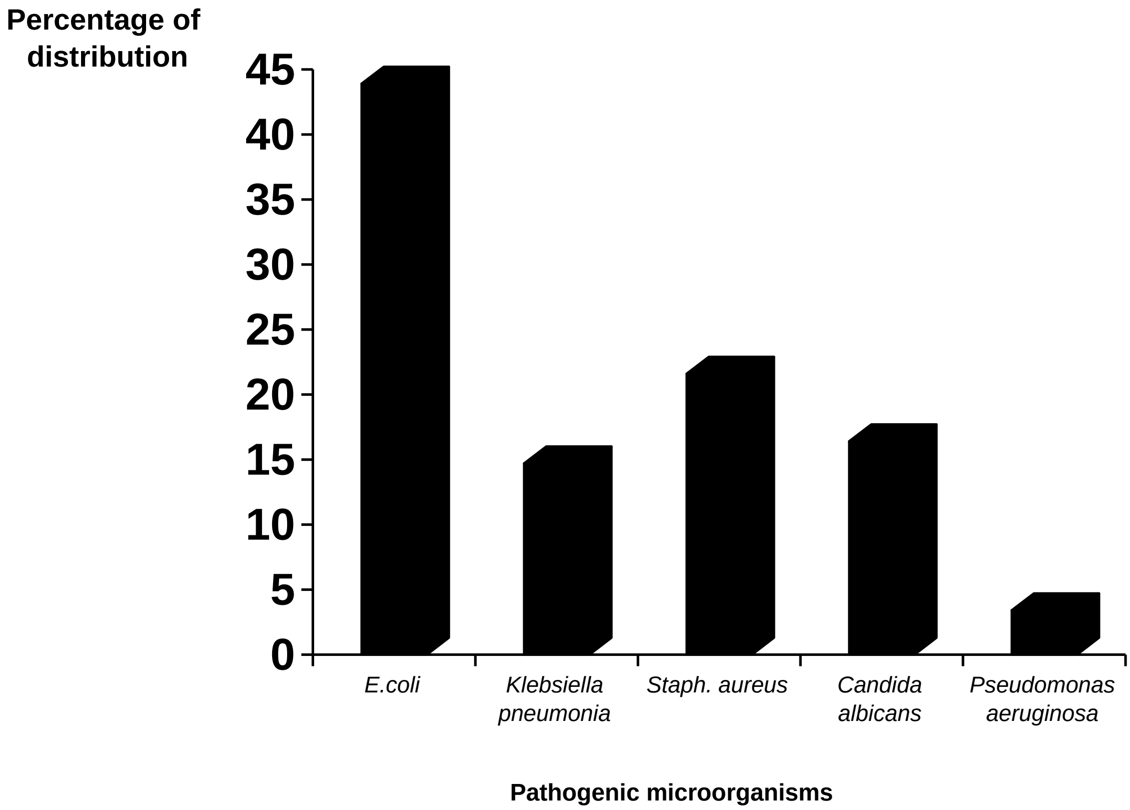
The results in Table (7) and Fig. (3, 4) indicated that the number of contaminated positive urine samples collected from males and females were 116 samples ,51 contaminated with *E. coli* , 25 of *Staph. aureus*, 19 *Candida albicans*, 17 of *Klebsiella pneumonia* and 4 of *Pseudomonas aeruginosae*.

So , the highest percentage of distribution are found in *E.coli* (43.9%) followed by *Staph. aureus* (21.6%), *Candida albicans* (16.4%) , *Klebsiella pneumonia* (14.7%) and *Pseudomonas aeruginosa* (3.4%).

Table (7) :The percentage of distribution number of pathogenic bacterial isolates and *Candida albicans* from positive collected samples.

Pathogenic isolates	Distribution number				Total isolates	Percentage of distribution (%)
	Male	%	Female	%		
E. coli	22	43.1	29	56.9	51	43.9
Klebsiella pneumonia	7	41.2	10	58.8	17	14.7
Staph. aureus	12	48	13	52	25	21.6
Candida albicans	8	42.1	11	57.9	19	16.4
Pseudomonas aeruginosa	1	25	3	75	4	3.4

$$\text{Percentage of distribution (\%)} = \frac{\text{No. of isolates of male or female}}{\text{Total no. of isolates}} \times 100$$



Fig(3):The percentage of distribution of pathogenic bacterial isolates and *Candida albicans* from positive collected samples.

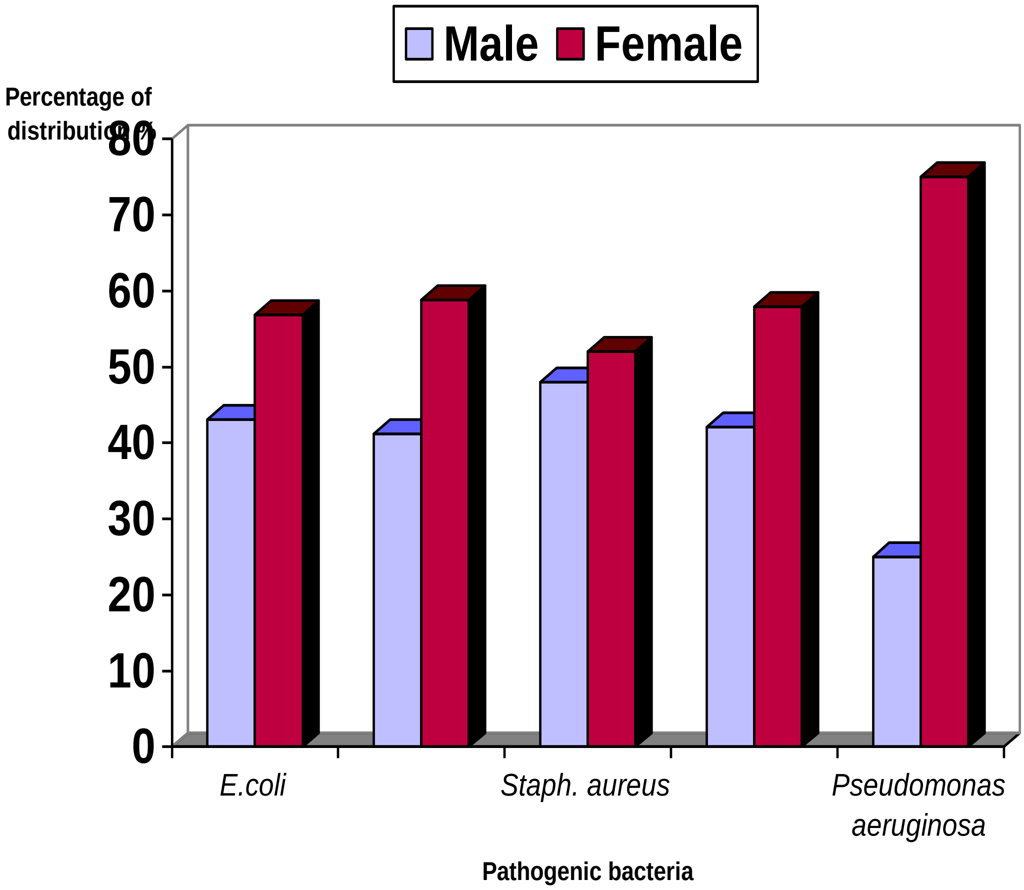


Fig (4)The percentage of distribution of pathogenic bacterial isolates and *Candida albicans* from collected positive samples in males and females.

5-The antagonistic effect of antibiotics drugs on isolated *E. coli*

Different commercial antibiotics were used to show their effect on isolated *E. coli* causing urinary tract infection in collected positive urine samples . the antibiotics used were amikacin (AK , 30µg) , amoxicillin – clavulanic acid (AMC, 30µg) , cefotaxime (CTX, 30µg) , ciprofloxacin (CIP, 5µg), gentamicin (CN, 10µg) , levofloxacin (LEV, 5µg) , nalidixic acid (NA, 30µg), norfloxacin (NOR, 10 µg) ,ofloxacin(OFX, 5µg) and sulbactam-ampicillin (SAM, 10µg) by using a standardized disc diffusion method.

The result in Table (8) revealed that:- The most effective antibiotics were ciprofloxacin , levofloxacin and norfloxacin followed by nalidixic acid , gentamicin and amikacin. Also the least effective one was sulbactam-ampicillin followed by amoxicillin-clavulanic acid and cefotaxime where ofloxacin show moderate effect.

The resistant *E. coli* isolates were samples numbers 1, 17, 19, 49, 54, 56 & 59 and the most resistant isolates numbers 19,59.The most sensitive *E. coli* isolates was number 102.

Table(8) Susceptibility and inhibition zone (mm) of different antibiotics drugs used against clinical *E.coli* isolates

	Susceptibility and inhibition zone (mm)																			
number of *E.coli*	**AMC (30 µg)**		**NOR (10 µg)**		**OFX (5 µg)**		**CTX (30 µg)**		**SAM (20 µg)**		**CIP (5 µg)**		**NA (30 µg)**		**LEV (5 µg)**		**AK (30 µg)**		**CN (10 µg)**	
	IZ	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**
1	0	R	21	S	0	R	11	R	0	R	24	S	0	R	23	S	16	I	14	I
3	0	R	21	S	0	R	13	R	0	R	25	S	23	S	24	S	18	S	15	S
11	15	I	29	S	30	S	13	R	0	R	30	S	23	S	30	S	19	S	18	S
12	17	I	28	S	0	R	0	R	0	R	26	S	20	S	26	S	17	S	15	S
17	0	R	27	S	0	R	14	R	0	R	23	S	30	S	25	S	16	I	0	R
19	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	20	S	0	R
20	0	R	27	S	0	R	7	R	0	R	29	S	24	S	27	S	19	S	16	S
25	0	R	27	S	0	R	0	R	0	R	30	S	25	S	30	S	20	S	18	S

Continous Table(8)

	Susceptibility and inhibition zone (mm)																			
number of *E.coli*	**AMC (30 µg)**		**NOR (10 µg)**		**OFX (5 µg)**		**CTX (30 µg)**		**SAM (20 µg)**		**CIP (5 µg)**		**NA (30 µg)**		**LEV (5 µg)**		**AK (30 µg)**		**CN (10 µg)**	
	IZ	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**
26	0	R	24	S	0	R	13	R	0	R	30	S	24	S	28	S	16	I	17	S
29	0	R	22	S	0	R	0	R	0	R	24	S	20	S	25	S	16	I	16	S
30	0	R	25	S	0	R	8	R	0	R	29	S	25	S	30	S	20	S	18	S
31	0	R	25	S	0	R	0	R	0	R	26	S	21	S	26	S	19	S	16	S
34	0	R	26	S	0	R	8	R	0	R	27	S	23	S	28	S	19	S	19	S
35	23	S	23	S	24	S	0	R	0	R	26	S	10	S	32	S	12	R	19	S
39	0	R	25	S	0	R	9	R	0	R	27	S	26	S	27	S	18	S	16	S
41	0	R	23	S	27	S	17	I	0	R	31	S	20	S	29	S	21	S	19	S

Continous Table(8)

	Susceptibility and inhibition zone (mm)																			
number of *E.coli*	**AMC (30 µg)**		**NOR (10 µg)**		**OFX (5 µg)**		**CTX (30 µg)**		**SAM (20 µg)**		**CIP (5 µg)**		**NA (30 µg)**		**LEV (5 µg)**		**AK (30 µg)**		**CN (10 µg)**	
	IZ	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**
45	0	R	21	S	0	R	12	R	0	R	22	S	20	S	25	S	16	I	16	S
49	0	R	23	S	0	R	10	R	0	R	24	S	25	S	25	S	13	R	13	I
52	0	R	22	S	0	R	11	R	0	R	27	S	23	S	23	S	16	I	15	S
54	0	R	25	S	0	R	14	R	0	R	25	S	23	S	26	S	16	I	0	R
56	0	R	26	S	0	R	12	R	0	R	25	S	22	S	25	S	16	I	9	R
57	0	R	24	S	0	R	0	R	0	R	26	S	21	S	27	S	19	S	16	S
59	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	15	I	0	R
61	0	R	30	S	29	S	7	R	0	R	31	S	23	S	30	S	21	S	18	S

Continous Table(8)

	Susceptibility and inhibition zone (mm)																			
Number of *E.coli*	**AMC (30 µg)**		**NOR (10 µg)**		**OFX (5 µg)**		**CTX (30 µg)**		**SAM (20 µg)**		**CIP (5 µg)**		**NA (30 µg)**		**LEV (5 µg)**		**AK (30 µg)**		**CN (10 µg)**	
	IZ	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**
63	0	R	28	S	30	S	0	R	0	R	31	S	21	S	30	S	21	S	17	S
65	0	R	28	S	27	S	0	R	0	R	27	S	21	S	28	S	21	S	17	S
67	0	R	26	S	32	S	0	R	0	R	31	S	22	S	31	S	22	S	20	S
69	0	R	31	S	31	S	0	R	0	R	33	S	22	S	33	S	23	S	21	S
70	0	R	26	S	31	S	0	R	0	R	30	S	19	S	29	S	19	S	17	S
73	0	R	25	S	32	S	9	R	0	R	30	S	25	S	31	S	21	S	19	S
75	0	R	24	S	29	S	0	R	0	R	27	S	22	S	29	S	21	S	18	S
76	0	R	27	S	35	S	0	R	0	R	23	S	24	S	31	S	25	S	22	S

Continous Table(8)

number of *E.coli*	Susceptibility and inhibition zone (mm)																			
	AMC (30 µg)		NOR (10 µg)		OFX (5 µg)		CTX (30 µg)		SAM (20 µg)		CIP (5 µg)		NA (30 µg)		LEV (5 µg)		AK (30 µg)		CN (10 µg)	
	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST	IZ	ST
77	0	R	27	S	30	S	0	R	0	R	30	S	21	S	30	S	21	S	19	S
80	0	R	26	S	29	S	10	R	0	R	28	S	20	S	24	S	19	S	17	S
82	0	R	22	S	28	S	0	R	0	R	27	S	22	S	27	S	20	S	17	S
84	0	R	26	S	27	S	0	R	0	R	28	S	19	S	28	S	19	S	17	S
86	0	R	20	S	27	S	0	R	0	R	26	S	19	S	26	S	20	S	16	S
89	0	R	30	S	30	S	0	R	0	R	29	S	24	S	29	S	21	S	19	S
90	11	R	25	S	0	R	12	R	0	R	27	S	26	S	30	S	19	S	17	S
91	0	R	29	S	34	S	11	R	0	R	35	S	23	S	33	S	24	S	21	S

Continous Table(8)

	Susceptibility and inhibition zone (mm)																			
number of *E.coli*	**AMC** (30 µg)		**NOR** (10 µg)		**OFX** (5 µg)		**CTX** (30 µg)		**SAM** (20 µg)		**CIP** (5 µg)		**NA** (30 µg)		**LEV** (5 µg)		**AK** (30 µg)		**CN** (10 µg)	
	IZ	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**
92	0	R	24	S	30	S	0	R	0	R	30	S	21	S	31	S	21	S	20	S
94	0	R	23	S	33	S	0	R	0	R	31	S	23	S	35	S	22	S	21	S
95	0	R	25	S	30	S	0	R	0	R	31	S	21	S	33	S	21	S	20	S
98	0	R	24	S	33	S	9	R	0	R	33	S	26	S	33	S	22	S	20	S
100	0	R	17	S	35	S	23	S	0	R	30	S	24	S	31	S	23	S	22	S
102	0	R	27	S	31	S	29	S	0	R	34	S	21	S	34	S	24	S	21	S
104	0	R	26	S	35	S	20	I	0	R	31	S	27	S	30	S	22	S	21	S
106	0	R	27	S	33	S	25	S	0	R	33	S	26	S	35	S	23	S	20	S

Continous Table(8)

	Susceptibility and inhibition zone (mm)																			
number of *E.coli*	**AMC (30 µg)**		**NOR (10 µg)**		**OFX (5 µg)**		**CTX (30 µg)**		**SAM (20 µg)**		**CIP (5 µg)**		**NA (30 µg)**		**LEV (5 µg)**		**AK (30 µg)**		**CN (10 µg)**	
	IZ	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**	**IZ**	**ST**
108	0	R	27	S	33	S	27	S	0	R	31	S	24	S	32	S	22	S	19	S
110	0	R	26	S	33	S	23	S	0	R	33	S	28	S	35	S	21	S	19	S
112	0	R	22	S	21	I	27	S	0	R	26	S	25	S	25	S	14	R	9	R

Susceptibility test = ST , **Inhibition zone(mm)** =IZ.

Sensitive = S , **Resistance** = R , **Intermediate** = I.

0 = No inhibition zone.

AMC= Amoxicillin – clavulanic Acid **, NOR=** Norfloxacin **, OFX=** Ofloxacin **, CTX=** Cefotaxime **, SAM=** Sulbactam-ampicillin

CIP= Ciprofloxacin **,** **NA=** Nalidixic acid , **LEV=** Levofloxacin **,** **AK=** Amikacin **,** **CN=** Gentamicin

6-Sensitivity test of pathogenic *E. coli* isolates against different tested antibiotics.

The sensitivity test of pathogenic *E. coli* isolates against different tested antibiotics were illustrated in Table (9).

These results showed that the antibiotics ciprofloxacin, norfloxacin and levofloxacin are more effective against isolated pathogenic *E. coli* which sensitivity reach to (96.1 %) followed by nalidixic acid(92.2%), gentamicin (84.3%) and amikacin (76.5%) .

The antibiotic amikacin showed intermediate effect against isolated pathogenic *E. coli* , which the percentage of intermediate reached to (17.6). On the other hand, the antibiotic sulbactam /ampicillin hasn't any effect against isolated pathogenic *E. coli* , where resistance reached to 100% followed by amoxicillin / clavulanic acid (94.1 %) and cefotaxime (84.3%).

Table(9) :Sensitivity test of pathogenic *E. coli* isolates against different antibiotics

Tested antibiotics	Sensitive isolates (S)		Intermediate isolates (I)		Resistant isolates (R)	
	No.	%	No.	%	No.	%
AMC	1	2	2	3.9	48	94.1
NOR	49	96.1	-	-	2	3.9
OFX	29	56.9	1	2	21	41.2
CTX	6	11.8	2	3.9	43	84.3
SAM	-	-	-	-	51	100
CIP	49	96.1	-	-	2	3.9
NA	47	92.2	-	-	4	7.8
LEV	49	96.1	-	-	2	3.9
AK	39	76.5	9	17.6	3	5.9
CN	43	84.3	2	3.9	6	11.8

7-Minimum inhibitory concentration (MIC) and minimum bactericidal concentration(MBC) of selected antibiotics:-

The experimental clinical *E. coli* were grown under stress of different concentrations of the selected antibiotics in nutrient broth and MICs and the MBCs were determined, as illustrated in Fig (5) .

The result in Table (10) indicated that the highest MIC was obtained when *E. coli* number 54 exposed to ciprofloxacin, gentamicin & and *E. coli* number 59 at ciprofloxacin, levofloxacin& norfloxacin antibiotics drugs which recorded 500 µg /ml and *E. coli* number 102 at ciprofloxacin. Lower MIC recorded 7.8 µg /ml for *E. coli* number 19& 49 at gentamicin & levofloxacin antibiotics drugs followed by *E. coli* number 1 at norfloxacin which recorded 15.63µg /ml . MICs equal MBCs for all selected antibiotics drugs.

Table (10):Minimum inhibitory concentration (MIC) and minimum bactericidal concentration(MBC) of some selected antibiotics.

E.coli isolates number	Amikacin		Ciprofloxacin		Gentamicin		Levofloxacin		Norfloxacin	
	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
1	125	125	250	250	250	250	62.5	62.5	15.63	15.63
17	125	125	15.63	15.63	250	250	125	125	62.5	62.5
19	62.5	62.5	125	125	31.25	31.25	7.8	7.8	62.5	62.5
49	250	250	62.5	62.5	7.8	7.8	250	250	15.63	15.63
54	250	250	500	500	500	500	125	125	500	500
56	125	125	125	125	125	125	62.5	62.5	62.5	62.5
59	31.25	31.25	500	500	31.25	31.25	500	500	500	500
102	125	125	500	500	31.25	31.25	62.5	62.5	31.25	31.25

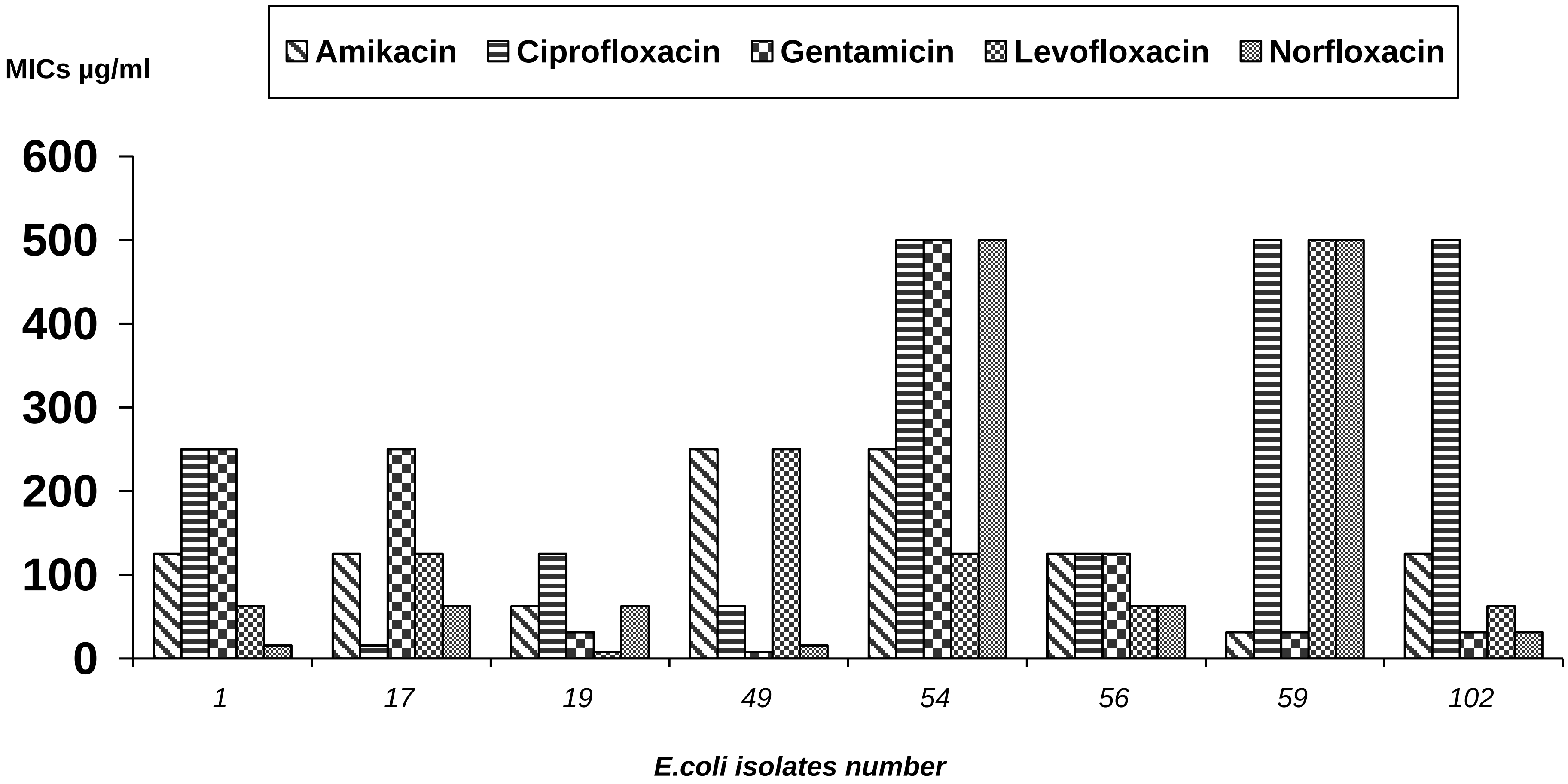


Fig.(5)Minimum inhibitory concentration (MICs)(µg/ml)of different antibiotics against tested *E.coli*

8-Effect of natural plant extract against selected isolates of *E. coli.*

In this experiment, an attempt was made to test the effect of different natural plant extracts (cinnamon, ginger (dry, fresh), spearmint, peppermint, marjoram, thyme, clove, lemon peel, orange peel, rosemary and oliban) ,as cold water extract, boiled water extracts and alcoholic extract against selected bacterial isolates *E. coli* number 1, 17, 19, 49, 54, 56, 59 and 102 as illustrated in Tables (11, 12 & 13).

8.a-Effect of alcoholic extracts :-

The results given in Table (11) showed that, the most effective plant extract was the alcoholic one of cloves with *E. coli* number19 which yield inhibition zone of 20 mm followed by peppermint with inhibition zone of 19mm in *E. coli* number 54 followed by rosemary with *E. coli* number 56 which recorded inhibition zone of 18mm followed by fresh ginger with *E. coli* number 1 & 59 with inhibition zone 16mm . On the other hand marjoram and oliban didn't show any effect against all selected isolates of *E. coli*. Alcoholic extract of rosemary has the lowest effect with *E. coli* number 49 which recorded inhibition zone of 7mm in diameter followed by ginger (dry) with *E. coli* 1 & 49 which yield inhibition zone of 8mm.

Table(11):- Effect of alcoholic plant extract on selected *E. coli* isolates.

	Diameter of inhibition zone (mm) of different alcoholic plant extract											
***E.coli* isolates number**	**Cinnamon**	**Dry ginger**	**Fresh ginger**	**Spearmint**	**Peppermint**	**Marjoram**	**Thyme**	**Clove**	**Lemon peel**	**Orange peel**	**Rosemary**	**oliban**
1	11	8	16	11	14	ND	12	18	13	13	ND	ND
17	9	12	11	13	14	ND	15	19	13	11	10	ND
19	12	11	12	9	15	ND	ND	20	12	10	11	ND
49	9	8	13	10	18	ND	18	19	9	13	7	ND
54	11	10	15	12	19	ND	10	18	15	14	ND	10
56	13	15	14	14	16	ND	14	11	14	13	18	ND
59	10	14	16	14	13	ND	15	14	12	14	12	ND
102	11	12	13	11	13	ND	9	16	15	14	ND	ND

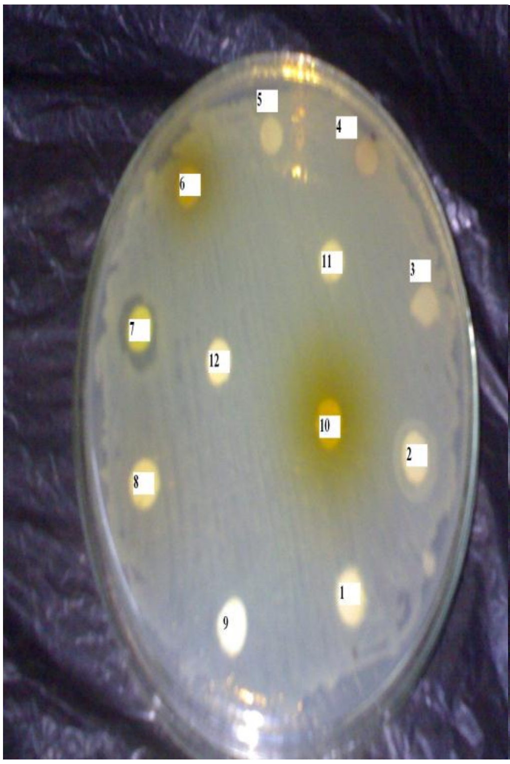
ND = Not detected(0.0)

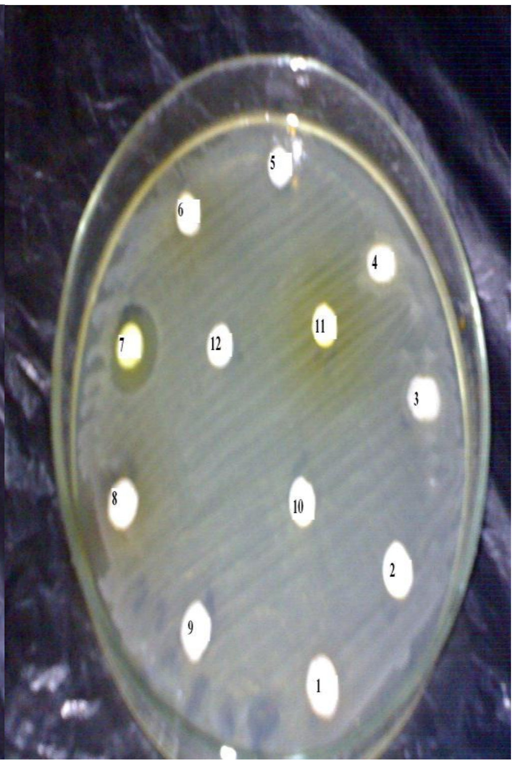
8.b-Effect of cold water extracts: The results given in Table(12) and photo number(1& 2) indicated that : All cold water extracts showed no effect against the selected *E. coli* isolates except thyme which showed great effect on all selected *E. coli* isolates .

Table(12):- Effect of cold water plant extract on selected *E. coli* isolates

	Diameter of inhibition zone (mm) of different cold water plant extract											
***E.coli* isolates number**	**Cinnamon**	**Dry ginger**	**Fresh ginger**	**Spearmint**	**Peppermint**	**Marjoram**	**Lemon peel**	**Clove**	**Thyme**	**Orange peel**	**Rosemary**	**Oliban**
1	ND	ND	ND	ND	ND	ND	ND	ND	16	ND	ND	ND
17	ND	ND	ND	ND	ND	ND	ND	ND	9	ND	ND	ND
19	ND	ND	ND	ND	ND	ND	ND	ND	12	ND	ND	ND
49	ND	ND	ND	ND	ND	ND	ND	ND	15	ND	ND	ND
54	ND	ND	ND	ND	ND	ND	ND	ND	20	ND	ND	ND
56	ND	ND	ND	ND	ND	ND	ND	ND	11	ND	ND	ND
59	ND	ND	ND	ND	ND	ND	ND	ND	12	ND	ND	ND
102	ND	ND	ND	ND	ND	ND	ND	ND	16	ND	ND	ND

ND= Not detected(0.0)



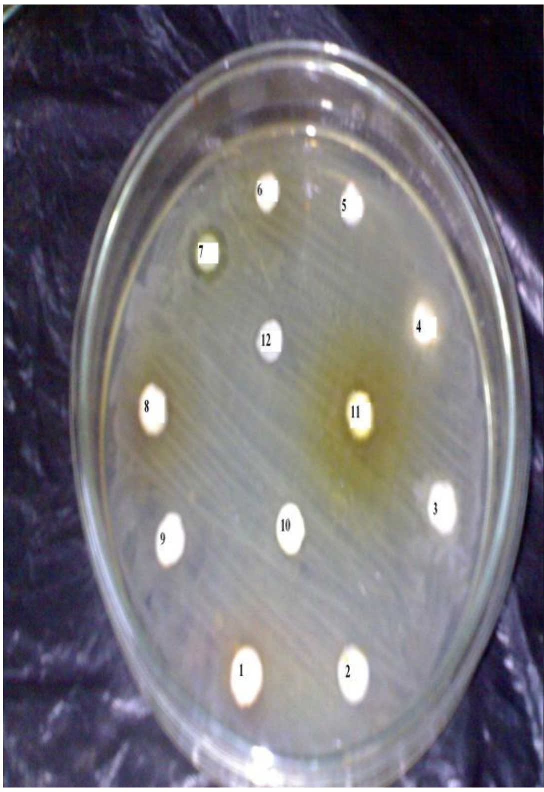


***E.coli* number 102** ***E.coli* number 59**

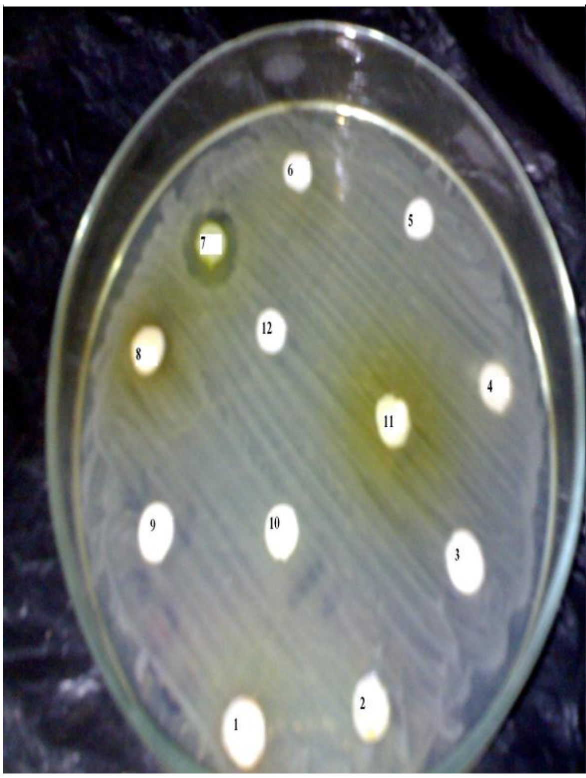
***E.coli* number 17** ***E.coli* number 1**

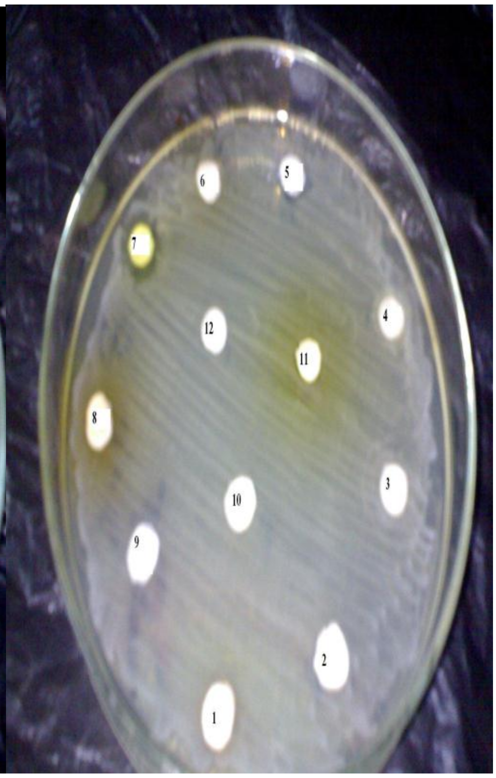
Photo No. (1) Effect of cold plant extract on the selected isolates no. (102, 59, 17&1)

1=Cinnamon 2=Ginger(Dry) 3=Ginger(Fresh) 4=Spearmint
5=Peppermint 6=Marjoram 7=Thyme 8=Clove
9=Lemon peel 10=Orange peel 11=Rosemary 12= Oliban.



***E.coli* number 54** ***E.coli* number 19**





***E.coli* number 56** ***E.coli* number 49**

Photo No. (2) Effect of cold plant extract on the selected isolates

1=Cinnamon 2=Ginger(Dry) 3=Ginger(Fresh) 4=Spearmint 5=Peppermint6=Marjoram 7=Thyme 8=Clove9=Lemon peel 10=Orange peel 11=Rosemary 12= Oliban.

8.c-Effect of boiling water extracts :the results in Table (13) showed that: The boiling water extracts of clove has an effect against selected *E. coli* isolates except with *E. coli* number 17. Boiling water extracts of rosemary and orange peel showed effect only against *E. coli* number 56. On the other hand cinnamon , ginger (fresh, dry), spearmint, peppermint, marjoram, thyme, lemon peel and oliban didn't show any effect against selected *E. coli* isolates.

Table(13):- Effect of boiled water plant extract on selected *E.coli* isolates.

	Diameter of inhibition zone (mm) of different boiled water plant extract											
***E.coli* isolates number**	**Cinnamon**	**Dry ginger**	**Fresh ginger**	**Spearmint**	**Peppermint**	**Marjoram**	**Thyme**	**Clove**	**Lemon peel**	**Orange peel**	**Rosemary**	**Oliban**
1	ND	ND	ND	ND	ND	ND	ND	9	ND	ND	ND	ND
17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	ND	ND	ND	ND	ND	ND	ND	11	ND	ND	ND	ND
49	ND	ND	ND	ND	ND	ND	ND	12	ND	ND	ND	ND
54	ND	ND	ND	ND	ND	ND	ND	10	ND	ND	ND	ND
56	ND	ND	ND	ND	ND	ND	ND	8	ND	10	7	ND
59	ND	ND	ND	ND	ND	ND	ND	9	ND	ND	ND	ND
102	ND	ND	ND	ND	ND	ND	ND	10	ND	ND	ND	ND

ND= Not detected(0.0).

9-Effect of different concentrations of natural plant extract against selected *E. coli* isolates.

Only five isolates of *E.coli* were selected according to their sensitivity to the plant extracts and then treated with different concentrations of alcoholic natural plant extract on nutrient medium and diameter of inhibition zones(mm) were measured.

9-a- The effect of different concentrations of alcoholic clove extract:

The most effective alcoholic plant extract as clove, fresh ginger, rosemary , peppermint and thyme and only water extract of thyme were selected to test the effect of different concentrations of these alcoholic and water extract on tested *E.coli* isolates as the following:

The obtained data recorded in Table (14) revealed that: The most effective concentrations of alcoholic extract of clove was 50% which yield inhibition zone of 20 mm and 10 mm in *E. coli* number 19 and *E. coli* number 17 respectively. The concentration 6.25% recorded lower inhibition zone 7 mm with *E. coli* number17 and *E. coli* number 54. Also concentration of 3.125% showed no effect with all selected *E. coli* isolates.

Table (14) Effect of different concentrations of alcoholic extract of clove on the selected *E. coli* isolates:-

Number of *E.coli* isolates	Inhibition zone (mm) of different conc. of alcoholic clove extract (%)				
	50	**25**	**12.5**	**6.25**	**3.125**
17	19	15	8	7	NI
19	20	NI	NI	NI	NI
49	17	15	NI	NI	NI
54	18	11	10	7	NI
102	16	16	12	NI	NI

NI: No inhibition zones.

% = Amount of extract complete to 100 ml of alcohol.

9-b-Effect of different concentrations of alcoholic fresh ginger extract:

Different concentrations of alcoholic fresh ginger were used as follows 50, 25, 12.5, 6.25, 3.125 % to study the effect of these concentrations on selected five *E .coli* isolates, the obtained results given in Table (15) showed that, the effective concentration of alcoholic extracts of ginger (fresh) against selected *E. coli* isolates was 50% which effected on all selected *E. coli* isolates. The concentration (25, 12.5, 6.25 and 3.125 %) showed no effect on all selected *E. coli* isolates.

Table (15):- Effect of different concentrations of alcoholic extract of fresh ginger on the selected *E. coli* isolates.

Number of *E.coli* isolates	Inhibition zone (mm) of different conc. of alcoholic fresh ginger extract (%)				
	50	25	12.5	6.25	3.125
17	15	NI	NI	NI	NI
19	12	NI	NI	NI	NI
49	12	NI	NI	NI	NI
54	14	NI	NI	NI	NI
102	13	NI	NI	NI	NI

NI: No inhibition zones.

% = Amount of extract complete to 100 ml of alcohol.

9-c-Effect of different concentrations of alcoholic rosemary extract:

The results illustrated in Table (16) showed that, the effective concentration of alcoholic extracts of rosemary against selected *E. coli* isolates was 50% which yield inhibition zone of 14 mm with *E. coli* number 54 and *E. coli* number 102. Concentrations of (12.5, 6.25 and 3.125 %) didn't show any effect against selected *E. coli* isolates.

Table (16) Effect of different concentrations of alcoholic extract of rosemary on the selected *E. coli* isolates:-

Number of *E. coli* isolates	**Inhibition zone (mm) of different conc. of alcoholic rosemary extract (%)**				
	50	**25**	**12.5**	**6.25**	**3.125**
17	10	NI	NI	NI	NI
19	11	NI	NI	NI	NI
49	12	7	NI	NI	NI
54	14	17	NI	NI	NI
102	14	NI	NI	NI	NI

NI: No inhibition zones.

% = Amount of extract complete to 100 ml of alcohol.

9-d-Effect of different concentrations of alcoholic peppermint extract:

The obtained data in Table (17) reveled that**,** the effective concentration of alcoholic extracts of peppermint against selected *E. coli* isolates was 50% which yield inhibition zone of 20 mm with *E. coli* number 17. Concentrations of 6.25% yield inhibition zone of 7 mm with *E. coli* number 49, 54, 102.

Table (17) Effect of different concentrations of alcoholic extract of peppermint on the selected *E. coli* isolates:-

Number of *E.coli* isolates	**Inhibition zone (mm) of different conc. of alcoholic peppermint extract (%)**				
	50	**25**	**12.5**	**6.25**	**3.125**
17	20	15	8	NI	NI
19	15	NI	NI	NI	NI
49	18	15	9	7	NI
54	17	14	8	7	NI
102	17	13	11	7	NI

NI: No inhibition zones.

% = Amount of extract complete to 100 ml of alcohol.

9-e-Effect of different concentrations of alcoholic thyme extract:

The results recorded in Table (18) revealed that, the effective concentration of alcoholic extracts of thyme against selected *E. coli* isolates was 50% which effected on all selected *E. coli* isolates and it recorded inhibition zone of 15 mm with *E. coli* number 17. Concentrations (12.5, 6.25 and 3.125 %) showed no effect on all selected *E. coli* isolates.

Table (18) Effect of different concentrations of alcoholic extract of thyme on the selected *E. coli* isolates:-

Number of *E. coli* isolates	Inhibition zone (mm) of different conc. of alcoholic thyme extract (%)				
	50	**25**	**12.5**	**6.25**	**3.125**
17	15	10	NI	NI	NI
19	11	NI	NI	NI	NI
49	14	NI	NI	NI	NI
54	15	14	NI	NI	NI
102	14	8	NI	NI	NI

NI: No inhibition zones.

% = Amount of extract complete to 100 ml of alcohol.

9-f-Effect of different concentrations of cold water extracts of thyme:

The obtained results in Table (19) showed that, the effective concentration of aqueous extracts of thyme against selected *E. coli* isolates was 50% which effected on all selected *E. coli* isolates. On the other hand Concentrations of (25, 12.5 , 6.25 and 3.125 %) didn't show any effect on all selected *E. coli* isolates.

Table (19) Effect of different concentrations of aqueous (cold) extract of thyme on the selected isolated of *E. coli*.

Number of *E.coli* isolates	Inhibition zone (mm) of different conc. of cold water extract of thyme (%)				
	50	**25**	**12.5**	**6.25**	**3.125**
17	9	NI	NI	NI	NI
19	12	NI	NI	NI	NI
49	15	NI	NI	NI	NI
54	20	NI	NI	NI	NI
102	16	NI	NI	NI	NI

NI: No inhibition zones.

% = Amount of extract complete to 100 ml of sterile distilled water.

10- Minimum inhibitory concentration (MIC) of selected plant extracts:

The results obtained in Tables number (14, 15, 16, 17,18 &19) indicated that the MIC of plant extracts (alcoholic &water) were recorded in Table (20) and Fig (6).

Lowest MIC was obtained when *E. coli* number 17 and 54 exposed to alcoholic extract of clove which recorded 6.25 % .Also when *E.coli* number 49, 54 and 102 exposed to alcoholic extract of peppermint.

Higher MIC recorded 50% with alcoholic extract of(clove, fresh ginger, rosemary, peppermint, thyme) and cold water extract of thyme for *E. coli* number 19.

Table (20) MICs of different natural plant extract on selected isolates of *Escherichia coli.*

	MIC of plant extract (%)					
Number of *E.coli* isolates	**Alcoholic clove**	**Alcoholic Fresh ginger**	**Alcoholic Rosemary**	**Alcoholic Peppermint**	**Alcoholic thyme**	**cold water thyme**
17	6.25	50	50	12.5	25	50
19	50	50	50	50	50	50
49	25	50	25	6.25	50	50
54	6.25	50	25	6.25	25	50
102	12.5	50	50	6.25	25	50

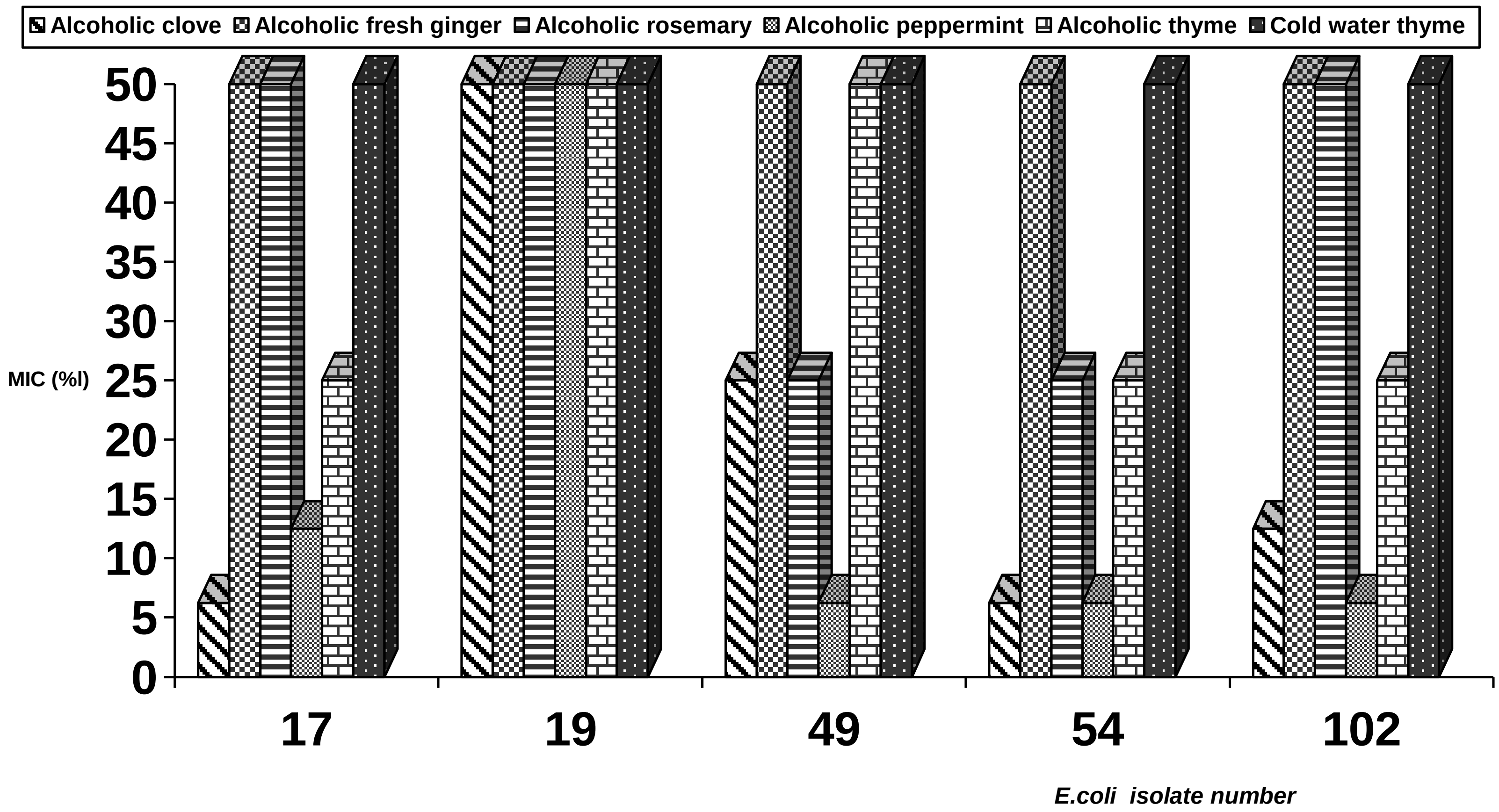


Fig.(6)Minimum inhibitory concentration (MICs)(%) of different plant extract against tested *E.coli*

11-Effect of combination between MIC of plant extracts and antibiotics on selected *E. coli* isolates.

The plants extracts that used in this experiment were clove, ginger (fresh), peppermint, thyme and rosemary which combined with MICs of amikacin, ciprofloxacin, gentamicin, levofloxacin and norfloxacin for each selected *E. coli* isolates to study the effect of combination between them and their action against *E. coli* isolates will increase than the effect of singly used or not as illustrated in photos number (3, 4, 5, 6 and 7).

Extraction of clove, ginger (fresh), peppermint and rosemary plant by alcohol while extraction of thyme by cold water and alcohol. These plant were used as it recorded the best extraction gave the highest antagonistic activities against selected *E. coli* isolates.

The results of combination effect were recorded in Tables(21, 22, 23, 24 and 25) and photos number (3, 4&5) .

11-a-Effect of MIC of amikacin combined with plant extract.

The results in Table (21) illustrated that the combination between MICs of amikacin antibiotics (with plant extract ginger "fresh" clearly synergestic action against *E. coli* number 17 and 49, synergism between plant extract rosemary and MICs of amikacin against *E. coli* number 54 and 102) more than singly used of antibiotic amikacin. On the other hand the antagonistic clearly illustrated with combination between clove, peppermint and thyme "cold water extract" and amikacin against *E.coli* isolates number 19 and 54 respectively which recorded inhibition action against these bacterial isolates less than singly used antibiotic.

11-b-Effect of MIC of ciprofloxacin combined with plant extract.

The obtained results in Table (22) indicated that, the combination between MICs of ciprofloxacin antibiotics with plant extract (clove, ginger "fresh", thyme (alcoholic)and rosemary clearly synergestic action against *E. coli* number 49 more than singly used antibiotic ciprofloxacin. On the other hand the antagonistic clearly illustrated with combination between MIC of ciprofloxacin with thyme(cold)& rosemary against *E. coli* number 17&19 which recorded inhibition zone against these bacterial isolates less than singly used antibiotics.

11-c-Effect of MIC of gentamicin combined with plant extract.

The obtained results in Table (23) indicated that, Synergistic effect of combination between MIC of gentamicin and clove, ginger "fresh", peppermint, thyme (alcoholic extract), thyme (cold water extract) and rosemary were recorded against *E. coli* number 49 which yield 24, 24, 34, 30, 26&35 mm respectively. Antagnostic effect were clearly obtained at combination of MIC of gentamicin with ginger "fresh"& thyme (cold water) against *E. coli* number 54.

11-d-Effect of MIC of levofloxacin combined with plant extract.

The results in Table (24) indicated that, Synergistic effect between MIC of levofloxacin and clove, peppermint, thyme(alcoholic, cold water extract) and rosemary were recorded against *E. coli* number 49 which yield 27, 31 ,34, 29 &30 mm) respectively. Antagnostic effect were clearly obtained in combination of MIC levofloxacin and thyme (alcoholic extract) against *E. coli* number 17.

11-e-Effect of MIC of norfloxacin combined with plant extract.

The results in Table (25) indicated that, synergistic effect of combination between MIC of norfloxacin with clove, ginger "fresh", peppermint, thyme(alcoholic extract) & rosemary against *E. coli* number 54. Antagonstic effect was obtained in combination of MIC of norfloxacin with ginger "fresh" and thyme (cold water extract) against *E. coli* number 56.Combination of MIC of norfloxacin with rosemary against *E .coli* number 54 , thyme (cold water extract) against *E. coli* number 102 didn't record any inhibition zone.

Table (21) Effect of combination between plant extracts and MICs of amikacin against selected *E. coli* isolates.

E. coli isolates number	Amikacin MICs		Diameter of inhibition zone (mm) of plant extract and antibiotic					
			Clove	Ginger "fresh"	Peppermint	Thyme alcoholic	Thyme cold water	Rosemary
	µg /ml	IZ	IZ	IZ	IZ	IZ	IZ	IZ
17	125	25	26	30	25	26	25	26
19	62.5	21	21	23	18	21	23	23
49	250	22	26	29	31	22	26	31
54	250	19	21	20	19	18	16	25
102	125	17	20	18	23	17	19	23

IZ= Inhibition zone.

Table (22) Effect of combination between plant extracts and MICs of ciprofloxacin against selected *E .coli* isolates.

E. coli isolates number	Ciprofloxacin MICs		Diameter of inhibition zone (mm) of plant extract and antibiotic					
			Clove	Ginger "fresh"	Peppermint	Thyme alcoholic	Thyme cold water	Rosemary
	µg /ml	IZ	IZ	IZ	IZ	IZ	IZ	IZ
17	15.63	30	30	31	30	30	25	28
19	125	12	20	12	12	13	11	10
49	62.5	27	29	24	32	30	30	37
54	500	10	13	15	11	20	NI	15
102	500	15	14	17	12	16	16	11

IZ= Inhibition zone.

NI :No inhibition zones at the concentration used.

Table (23) Effect of combination between plant extracts and MICs of gentamicin against selected *E. coli* isolates.

E.coli isolates number	Gentamicin MICs		Diameter of inhibition zone (mm) of plant extract and antibiotic					
			Clove	Ginger "fresh"	Peppermint	Thyme alcoholic	Thyme cold water	Rosemary
	µg /ml	IZ	IZ	IZ	IZ	IZ	IZ	IZ
17	250	31	25	26	25	33	31	23
19	31.25	15	18	16	13	21	19	12
49	7.8	19	24	24	34	30	26	35
54	500	16	18	11	19	20	12	18
102	31.25	13	24	12	19	17	16	12

IZ= Inhibition zone.

Table (24) Effect of combination between plant extracts and MICs of levofloxacin against selected *E.coli* isolates.

E.coli isolates number	Levofloxacin MICs		Diameter of inhibition zone (mm) of plant extract and antibiotic					
			Clove	Ginger "fresh"	Peppermint	Thyme alcoholic	Thyme cold water	Rosemary
	µg /ml	IZ	IZ	IZ	IZ	IZ	IZ	IZ
17	125	30	31	25	32	7	31	30
19	7.8	20	12	16	14	18	21	11
49	250	26	27	20	31	34	29	30
54	125	20	20	15	20	16	18	17
102	62.5	18	19	17	17	19	19	15

IZ= Inhibition zone.

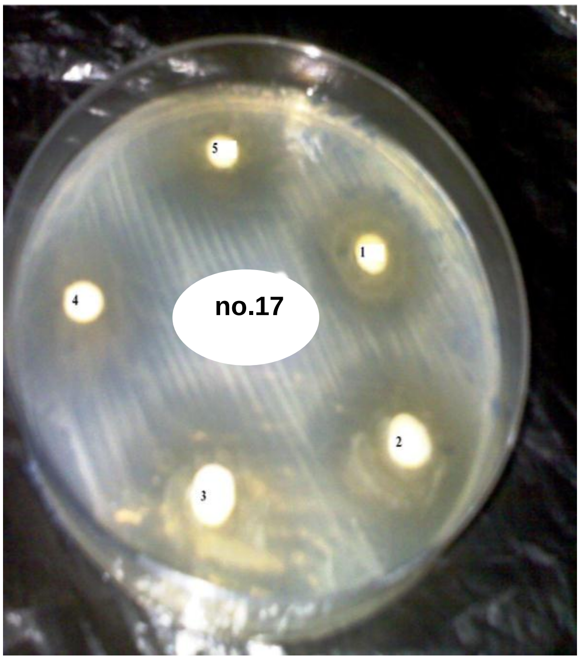
NI :No inhibition zones at the concentration used.

Table (25) Effect of combination between plant extracts and MICs of norfloxacin against selected *E.coli* isolates.

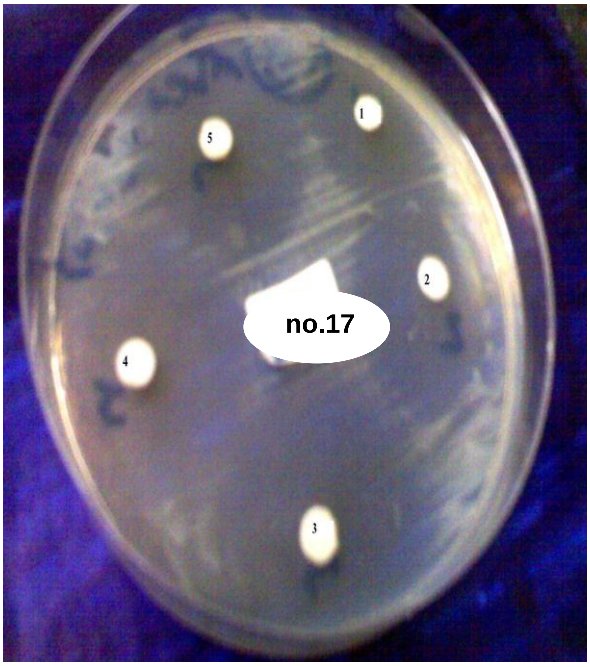
E.coli isolates number	Norfloxacin MICs		Diameter of inhibition zone (mm) of plant extract and antibiotic					
			Clove	Ginger "fresh"	Peppermint	Thyme alcoholic	Thyme cold water	Rosemary
	µg /ml	IZ	IZ	IZ	IZ	IZ	IZ	IZ
17	62.5	26	25	29	26	25	27	28
19	62.5	8	14	15	14	7	17	20
49	15.63	30	35	26	35	30	26	32
54	500	7	16	9	18	14	16	NI
102	31.25	9	21	13	16	18	NI	17

IZ= Inhibition zone.

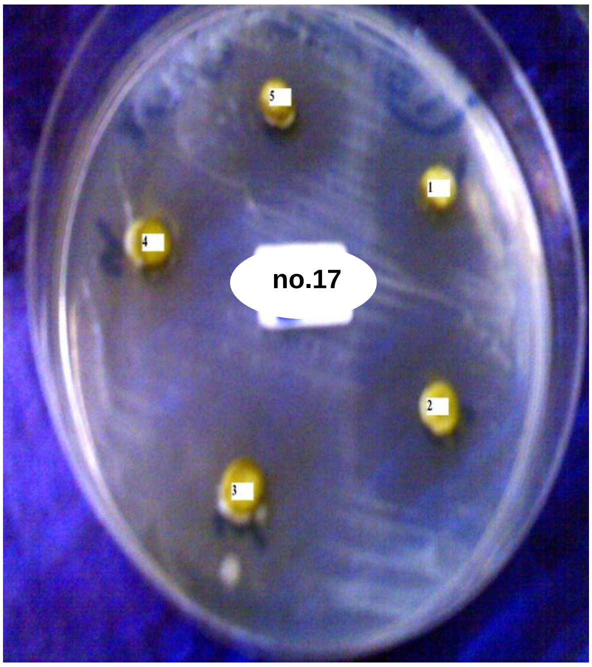
NI :No inhibition zones at the concentration used.



MIC+ clove for *E.coli* 17



MIC+ thyme (aqueous) for *E.coli* 17

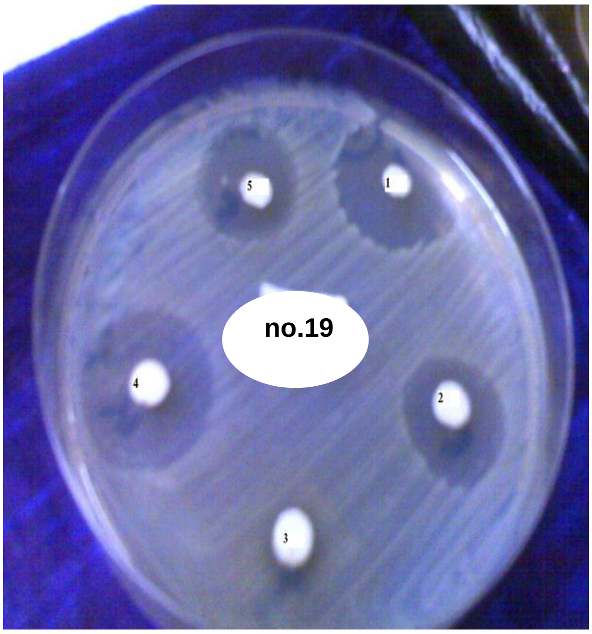


MIC+ rosemary for *E.coli* 17

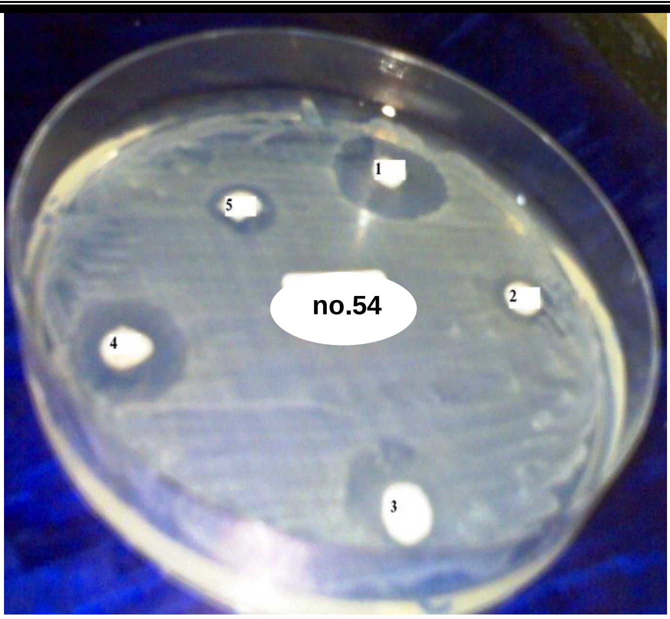
1=Amikacin 2- Norfloxacin 3=Ciprofloxacin 4=Levofloxacin 5=Gentamicin

Photo No. (3)Effect of combination between antibiotic and plant extracts against bacterial isolates no. 17 by disc diffusion method

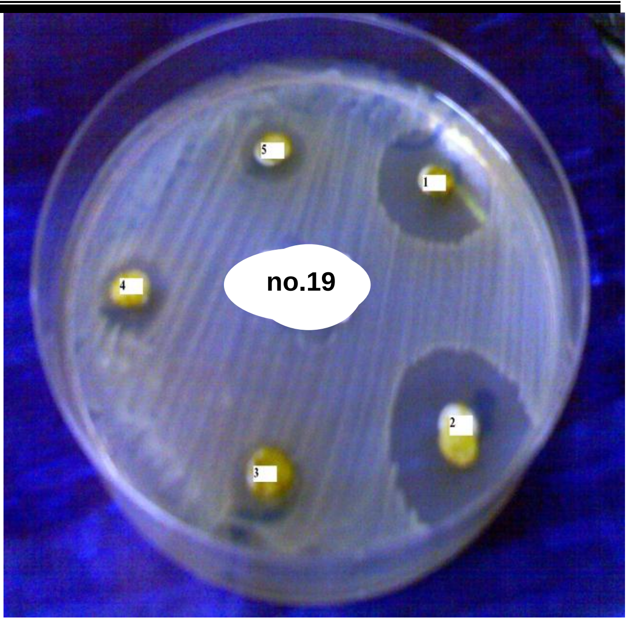
MIC+ginger fresh for *E.coli* 19



MIC+thyme (aqueous) for *E.coli* 19



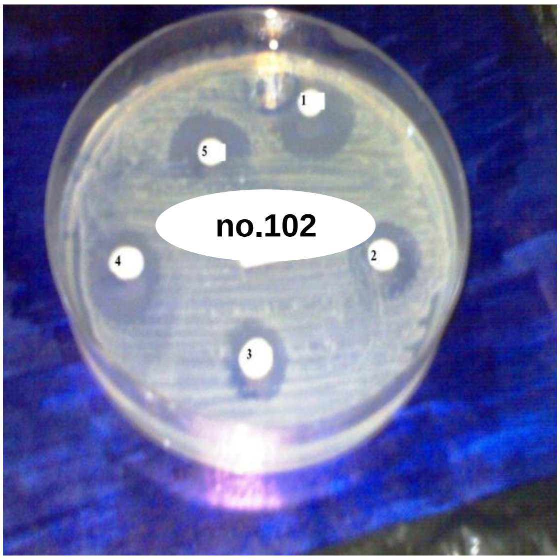
MIC+ginger fresh for *E.coli* 54

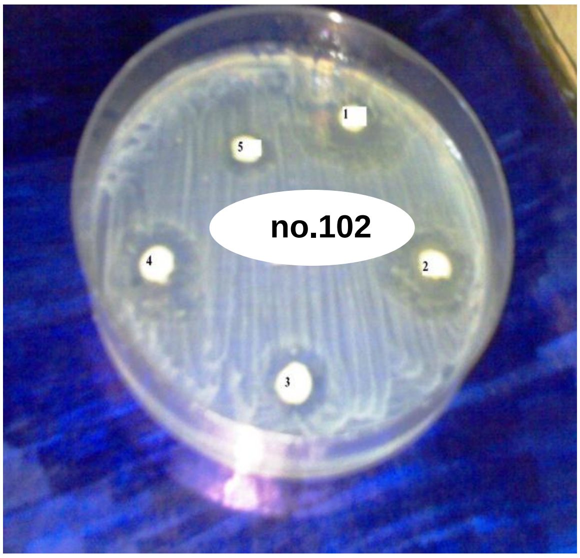


MIC+rosemary for *E.coli* 19

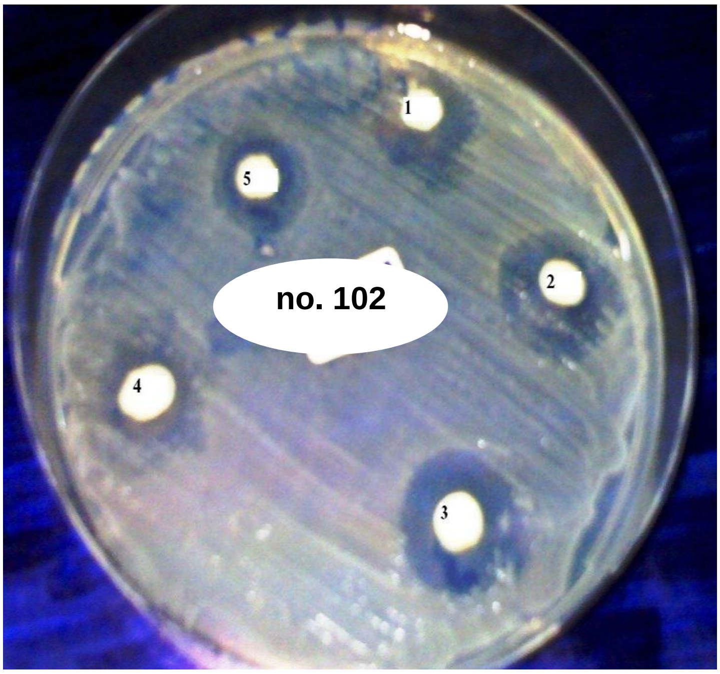
1=Amikacin 2- Norfloxacin 3=Ciprofloxacin 4=Levofloxacin 5=Gentamicin

Photo No. (4)Effect of combination between antibiotic and plant extracts against bacterial isolates no. 19&54 by disc diffusion method





MIC+peppermint for *E.coli* 102 **MIC+thyme alcoholic for *E.coli* 102**



MIC+thyme alcoholic for *E.coli 102*

1=Amikacin 2- Norfloxacin 3=Ciprofloxacin 4=Levofloxacin 5=Gentamicin

Photo No. (5)Effect of combination between antibiotic and plant extracts against bacterial isolates no. 102 by disc diffusion method

12- Protein analysis for clinical bacterial isolates under stress of clove extract.

The experimental clinical bacterial isolates *E.coli* number 19, 54 & 102 were grown on nutrient agar without any stress at normal condition as control samples, and also grown on nutrient agar under stress of concentrated alcoholic extract of clove for each selected isolates *E. coli* as treated samples.

The result in photo number(6) indicated that there were difference in protein bands between bacteria before and after treatment, where in case of *E. coli* number 19 the molecular weight for each protein bands were (100, 98,95, 68 and 60) KD while when treated with clove the molecular weight 100, 98 and 95 KD was disappear. In *E. coli* number 54 the molecular weight for protein bands were (100, 98, 95, 68 and 60) KD . On the other hand when treated with clove the molecular weight of 100 and 98 KD were disappear, and in *E. coli* number 102 the molecular weight for each protein bands were (68, 60) KD while when treated with clove the molecular weight for protein band was 60 KD.

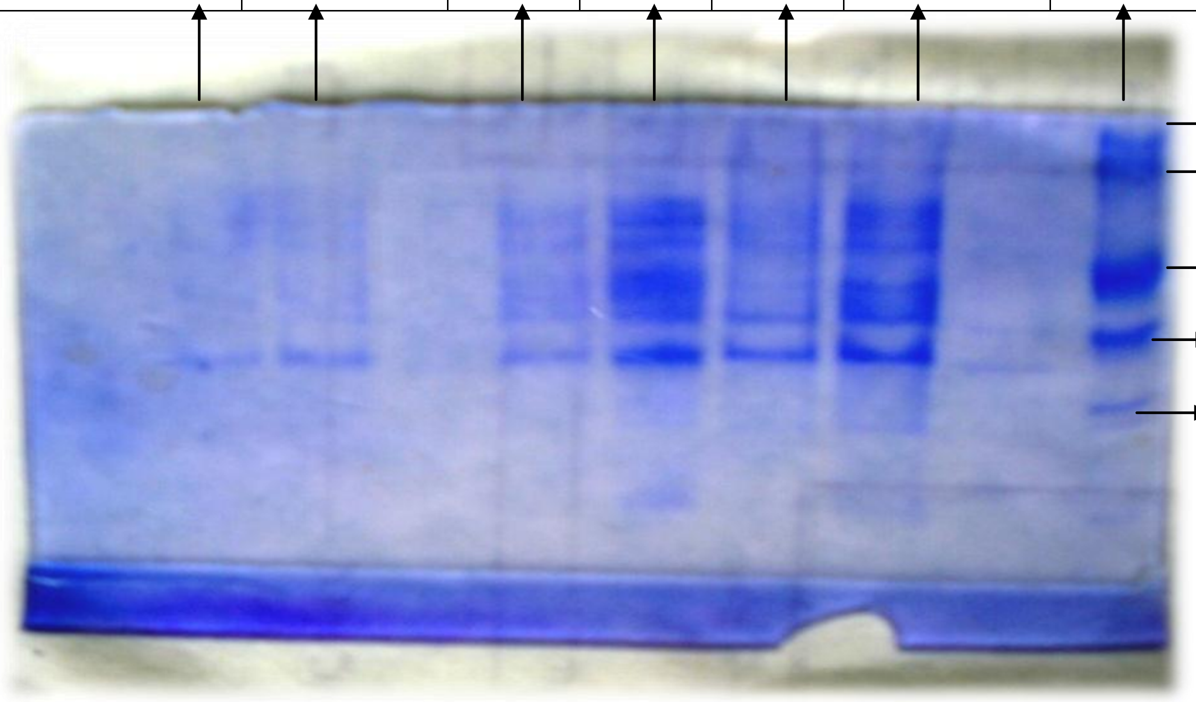
Lan6	Lan5	Lan4	Lan3	Lan2	Lan1	M	K.Da
							205 116 97 66 45

Photo No. (6): Protein analysis for clinical *E .coli* before and after stress by clove extract.

M=Marker (Molecular weight of marker)

Band (1)205:band(2):116:band(3)97:band(4)66: band(5) 45 .

Lan 1- *E.coli* No.(19)before stress(control).

Lan 2-*E.coli* No.(19) after stress.

Lan 3- *E.coli* No.(54) before stress(control).

Lan 4- *E.coli* No.(54) after stress.

Lan 5- *E.coli* No.(102) before stress(control).

Lan 6- *E.coli* No.(102) after stress.

13- Purification and identification of clove antibacterial extracted compound:

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

RF number 10 zone gave the antibacterial activities with all bacterial isolated from patients with urinary tract infection. While the rest RF showed no activity against tested *E. coli*.

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

a-^{1}HNMR spectrum:

^{1} HNMR of compound showed signals at 3.8 (d, 2H,CH2), 5(t, 2H, =CH2), 6(m, 1H, -CH=), 6.5-6.8(m, 3H,Ar H^{5}s) and at 8.6(S, 1H, OH); as illustrated in Fig.(6).

b- IR spectrum:

IR of compound showed broad band at 3500 cm-1corresponding to –OH group and band at 1518 cm-1corresponding to C=C, in addition to O-C band absorption at 1099 cm-1; as illustrated in Fig. (7and 8).

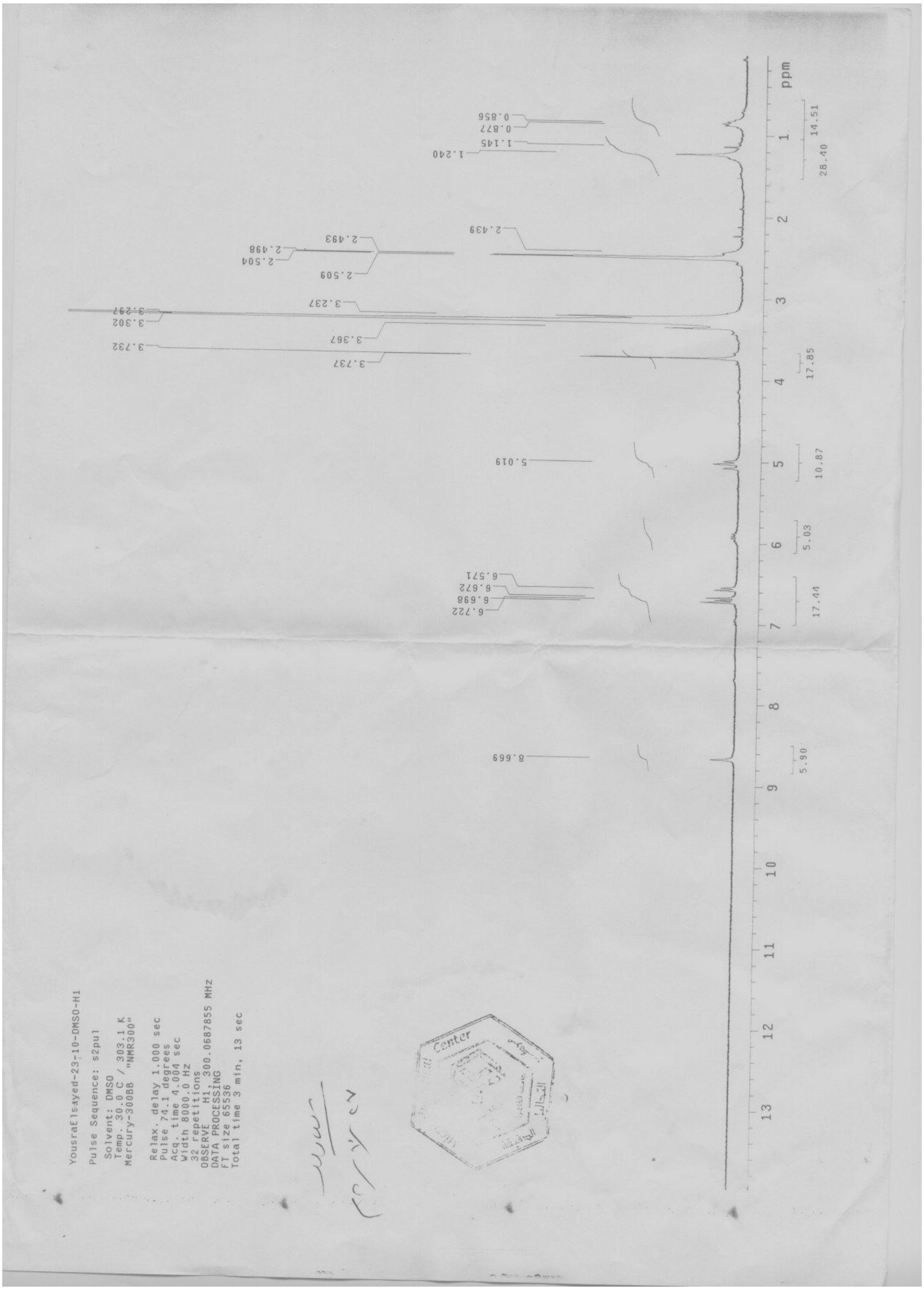
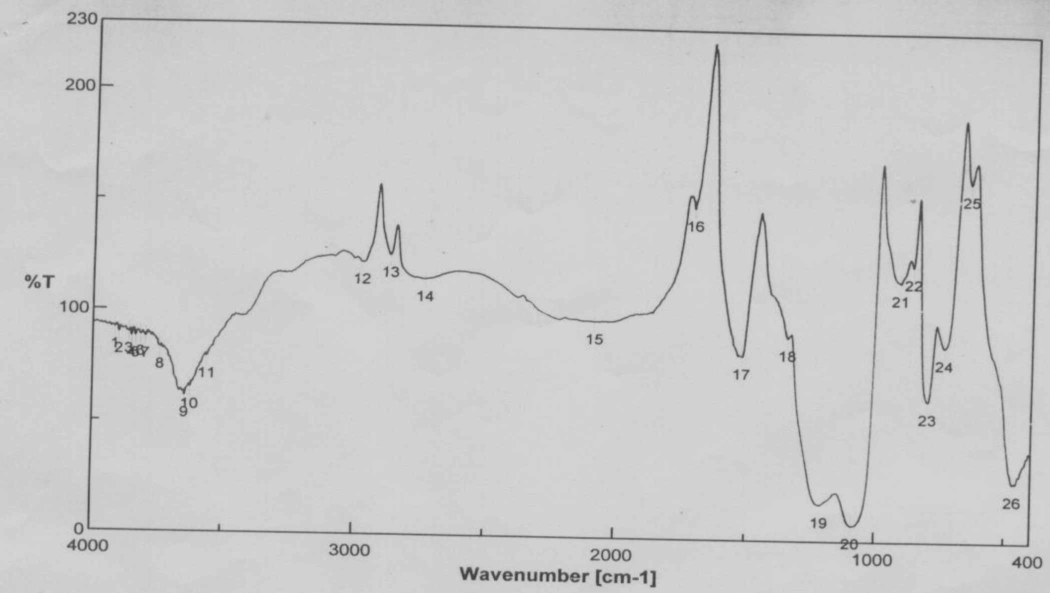


Fig. (7): [1] HNMR spectrum of antimicrobial substances.



Accumulation	16
Resolution	4 cm-1
Zero Filling	ON
Apodization	Cosine
Gain	Auto (4)
Scanning Speed	Auto (2 mm/sec)
Date/Time	20/10/08 11:36Ö
Update	28/10/08 6:36Ö
Operator	
File Name	Memory#13
Sample Name	SAMP. + SIL. GEL. – Si Gell
Comment	

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3916.72	91.9455	2	3900.32	89.8259	3	3861.75	89.8089
4	3851.15	88.0748	5	3836.68	88.1248	6	3818.36	88.8063
7	3799.08	88.004	8	3743.15	83.2303	9	3647.7	61.5916
10	3627.45	65.4264	11	3564.77	79.2783	12	2980.45	123.065
13	2873.42	126.367	14	2739.39	115.764	15	2087.57	97.837
16	1715.37	149.633	17	1524.45	83.2173	18	1348.96	91.8472
19	1213.01	16.7138	20	1087.66	7.38467	21	915.058	117.49
22	864.917	124.814	23	800.314	63.8698	24	740.531	88.2972
25	649.893	162.721	26	468.617	26.9565			

Fig (8): IR spectrum of the antimicrobial substances

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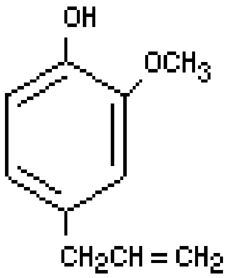
All data corresponding to eugenol structure.



Eugenol