RESULTS

1- Isolation of selected organisms

It has taken seventy-eight urine samples from diabetic patients isolated from different ages of males and females ranged 30-70 years of males and 35-60 years of females.

We found that 50 out of 78 samples of them were positive (in presence of E. *coli, K. aerogenes, P. aeroginosae*) and twenty eight samples were negative (no presence of *E. coli, K. aerogenes, P. aeroginosae*). The highest percentage rate obtained was 85.71% of positive samples found in male age from 61-70 years and the highest percentage rate of female samples reach to 83.33% at ages 46-50 years as illustrated in table (1) and fig. (1).

Statistical analysis of results obtained in table (2) illustrated that the results are significant if compared with the age of patients, sexes (male and female) and personal state, but these results not significant with insulin dependent or independent.

Table (1) : Diabetic urine sample collected from different ages of male and female (positive samples contaminated with *E. coli* or *K. aerogenes, P. aeroginosae*).

Source of samples	Total samples	Positive samples	Percentage, (%)	Dependent insulin		Personal states	
				Dependent	Independent	Single	Married
Age of males (years)							
30-35	12	7	58.33	3	9	2	10
36-40	8	5	62.50	2	6	3	5
41-45	3	1	33.33	1	2	-	3
46-50	6	5	66.66	3	3	2	6
51-55	5	4	80.00	2	3	-	5
56-60	7	5	71.42	3	4	1	6
61-70	7	6	85.71	2	5	-	7
Age of females (years)							
35-40	8	3	37.5	3	5	2	6
41-45	5	2	40	3	2	1	4
46-50	6	5	83.33	2	4	-	6
51-55	4	3	75	-	4	-	4
56-60	7	4	57.14	-	7	-	7
	78	50					

$$\text{Percentage} = \frac{\text{Positive samples}}{\text{Total samples}} \text{ X } 100$$

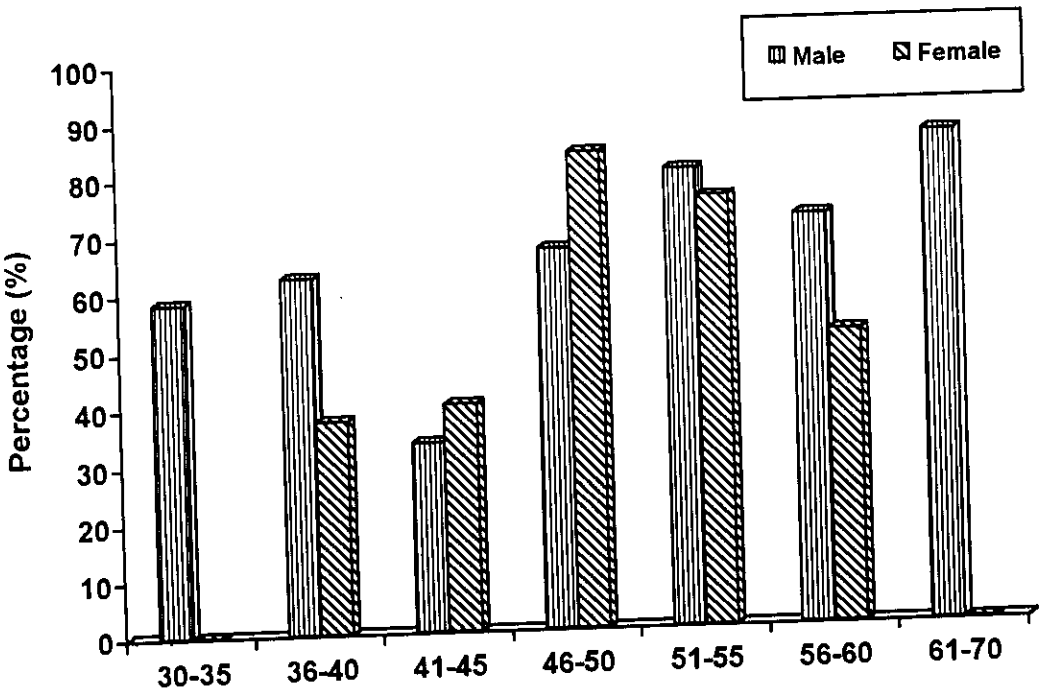


Fig. (1) : Diabetic urine sample collected from different ages of male and female (positive samples contaminated with *E. coli* or *K. aerogenes, P. aeroginosae*).

Table (2) : Statistical analysis and characteristics of the studied groups.

Characters	Positive samples		Negative samples		Test of significant	P
Age						
$\bar{X} \pm SD$	51.2 ± 10.3		48.5 ± 11.2		T	0.28
Range	32-82		30-72		1.08	NS
Gender	No	%	No	%	X^2	P
Male	32	64.0	19	67.9	0.12	0.73
Female	18	36.0	9	32.1		NS
Social states						
Lower	24	48.0	9	32.1	1.85	0.17
Middle	26	52.0	19	67.9		NS
Personal states						
Single	12	24.0	6	21.4	0.07	0.79
Married	38	76.0	22	78.6		NS
Insulin						
+ ve	35	70.0	20	71.4	0.02	0.89
- ve	15	30.0	8	28.6		NS

No. = Number

X^2 = Test of significance (Chi - square)

P = Probability

P > 0.05 = Non significant

P < 0.05 = Significant

P < 0.01 = Highly significant

NS = Non significant

T = Test of significant

2- Identification of bacterial isolates

Three bacterial specific *E. coli* or *K. aerogenes*, and *P. aeroginosae* were isolated from urine samples of diabetes mellitus.

The clinical isolates were subjected to two patterns of identification according to the Bregey's Manual of Determinative Bacteriology **(Le Minor, 1984 and Rowe and Gross 1984).**

First the straining reactions and culture characteristics of the isolates on simple, enriched and selective media as well as biochemical reactions.

K. aerogenes and *E. coli* are Gram-negative, actively motile bacilli, while *P. aeroginosae* are non-motile Gram-negative bacilli. The isolates were identified biochemically through the following biochemical reaction ; lactase fermentation, H_2S production, urease production, sugars fermentation, indole production, phenylalanine deaminase , methyl red, Voges-Proskauer and oxidase enzyme production.

Table (3) showed that the previous biochemical reaction for each species as following ; *E. coli* isolate were motile, Gram-negative, form acid and gas from glucose, ferment lactose, produce indole, gives positive methyl-red reaction and negative Voges-Proskauer reaction, decompose urea, produce H_2S and oxidase enzyme and utilize citrate but not reduced nitrate, while *K. aerogenes* isolates were motile, Gram-negative, bacilli, non-lactose fermeneters, form only acid from glucose, produce urease, gives positive methyl-red reaction and negative Voges-Proskauer reaction, produce H_2S but don't produce oxidase enzyme and utilize citrate and reduced nitrate.

produce oxidase enzyme but don't produce H_2S and utilize citrate but not reduced nitrate.

Table (3) : Biochemical reaction of *E. coli*, *K. aerogenes* and *P. aeroginosae*.

Isolate reaction	*E. coli*	*K. aerogenes*	*P. aeroginosae*
H_2S	Positive	Positive	Negative
Lactase	Positive (A/G)	Negative	Negative
Glucose	Positive (A/G)	Positive (A)	Positive
Maltose	Positive (A/G)	Positive (A)	Negative
Sucrose	Positive (A/G)	Positive	Negative
Indole	Positive	Negative	Positive
Urease	Negative	Positive	Negative
Motility	Positive	Positive	Negative
VP	Negative	Negative	Negative
Oxidase	Positive	Negative	Positive
Mannitole	Positive (A/G)	Positive (A)	Negative
Citrat utilization	Positive	Positive	Positive
MR	Positive	Positive	Positive
Nitrate reduction	Negative	Positive	Negative

A = Acid producer

G = Gas producer

VP = Voges-Proskauer

MR=Methyle Red test

3- The distribution of pathogenic isolate from positive urine samples

The results in table (4) and fig. (2) indicate that the number of contaminated positive diabetic urine samples collected from males and females were 29 samples contaminated with *E. coli*, 13 of *K. aerogenes* and 8 of *P. aeroginosae* from positive collected samples. The highest percentage of distribution were found in *E. coli* (58%) followed by *K. aerogenes* (26%) and *P. aeroginosae* (16%).

Table (4) : The distribution number of pathogenic bacterial isolate from positive collected samples.

Pathogenic bacterial isolates	Total samples	Positive samples	Distribution number				Percentage of distribution, (%)
			Male	%	Female	%	
E. coli	50	29	21	65.6	8	44.4	58
K. aerogenes	50	13	11	34.32	2	11.1	26
P. aeroginosae	50	8	5	15.61	3	16.6	16

$$\text{Percentage of distribution} = \frac{\text{Positive samples}}{\text{Total samples}} \text{ X } 100$$

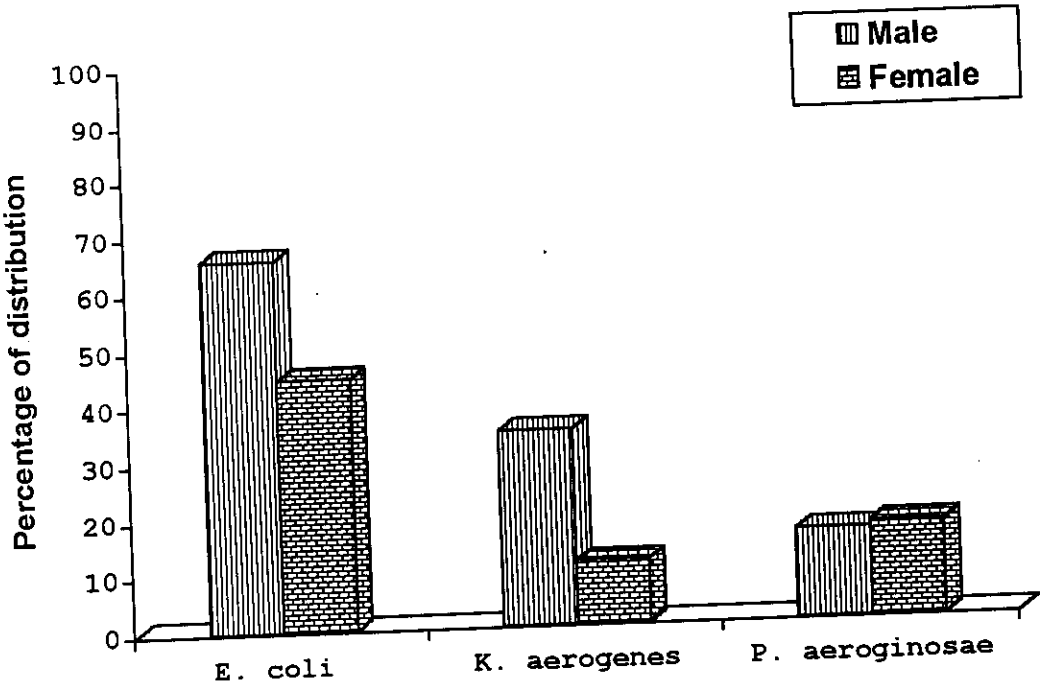


Fig. (2) : The distribution number of pathogenic bacterial isolate from positive collected samples.

4- The effect of different antibiotics on pathogenic isolates

Different commercial antibiotics used to show their effect on pathogenic isolated bacterial organisms causing urinary tract infection in diabetic urine collected samples.The antibiotics used were cephradine (10 μg), ampicillin (25μg), septrin sulfatrimero (25 μg), noroxin (10 μg), amikin (10 μg), nitrofurrantion (30 μg) using a standardized disc diffusion method.

The results in table (5) Plate (1,2,3) revealed that the six isolates of *E. coli* sensitive to cephradine, five isolates sensitive to ampicillin, six isolates sensitive to septrin sulfatrimero, seventeen isolates sensitive to noroxin, five isolates sensitive to amikin, twenty four isolates sensitive to nitrofurrantion.

The *E. coli* isolate number twenty-four is more sensitive to nitrofurrantion and *E. coli* isolate number five is more resistance to ampicillin and amakin,the same results indicate that the seven isolates of *K. aerogenes* sensitive to cephradine, five sensitive to ampicillin, nine sensitive to septrin sulfatrimero, nine sensitive to noroxin, five sensitive to amikin, six sensitive to nitrofurrantion.

These results also revealed that the two isolates of *P. aeroginosae* sensitive to cephradine, two sensitive to ampicillin, one sensitive to septrin sulfatrimero, four sensitive to noroxin, three sensitive to nitrofurrantion and the *P. aeroginosae* number 4 is more sensitive to noroxin.

Table (5) : Inhibition zone (mm) of different pathogenic bacterial isolates against different tested antibiotics.

No	Pathogenic isolates	Cephradine (10 µg)		Ampicillin (25 µg)		Septrin sulfatrimero (25 µg)		Noroxin (10 µg)		Amakin (10 µg)		Nitrofurran-tion (30 µg)	
		IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S
1	*E. coli*	19	S	20	S	12	R	12	R	12	R	20	S
2	*E. coli*	14	S	11	R	9	R	30	S	12	R	20	S
3	*K. aerogenes*	24	S	32	S	30	S	32	S	8	R	12	R
4	*E. coli*	10	R	ND		11	R	29	S	12	R	18	S
5	*E. coli*	11	R	ND		12	R	11	R	ND		20	S
6	*P. aeroginosae*	19	S	20	S	11	R	30	S	10	R	20	S
7	*P. aeroginosae*	ND		ND		ND		30	S	11	R	ND	
8	*K. aerogenes*	ND		ND		32	S	10	R	9	R	21	S
9	*E. coli*	12	R	13	R	8	R	30	S	ND		20	S
10	*K. aerogenes*	12	R	12	R	11	R	32	S	ND		10	R
11	*E. coli*	16	S	8	R	11	R	9	R	12	R	21	S
12	*P. aeroginosae*	11	R	18	S	29	S	30	S	ND		21	S
13	*E. Coli*	12	R	12	R	29	S	12	R	11	R	19	S
14	*K. aerogenes*	8	R	10	R	30	S	30	S	31	S	20	S
15	*E. coli*	11	R	11	R	12	R	11	R	10	R	19	S
16	*E. coli*	12	R	8	R	12	R	30	S	ND		21	S
17	*K. aerogenes*	16	S	12	R	8	R	30	S	ND		11	R
18	*P. aeroginosae*	ND		ND		ND		11	R	12	R	ND	
19	*E. coli*	11	R	13	S	11	R	8	R	11	R	20	S
20	*K. aerogenes*	10	R	22	S	30	S	10	R	10	R	20	S
21	*E. coli*	ND		ND		ND		ND		10	R	ND	
22	*E. coli*	11	R	ND		12	R	30	S	12	R	20	S

23	*P. aeroginosae*	ND		ND		10	R	11	R	12	R	ND			
24	*P. aeroginosae*	21	S	11	R	12	R	31	S	ND		20	S		
25	*K. aerogenes*	22	S	29	S	25	S	30	S	11	R	12	R		
26	*E. coli*	11	R	18	S	12	R	29	S	10	R	19	S		
27	*P. aeroginosae*	ND		ND		ND		12	R	8	R	ND			
28	*E. coli*	12	R	10	R	9	R	11	R	ND		17	S		
29	*E. coli*	14	S	10	R	29	S	30	S	11	R	21	S		
30	*P. aeroginosae*	ND		ND		ND		ND		12	R	ND			
31	*E. coli*	12	R	18	S	12	R	12	R	23	S	20	S		
32	*K. aerogenes*	10	S	ND		31	S	11	R	30	S	21	S		
33	*E. coli*	ND		ND		ND		11	R	30	S	21	S		
34	*E. coli*	11	R	12	R	10	R	ND		12	R	21	S		
35	*K. aerogenes*	20	S	3	S	12	R	30	S	24	S	12	R		
36	*E. coli*	ND		ND		ND		30	S	10	R	ND			
37	*K. aerogenes*	12	R	ND		30	S	30	S	8	R	19	S		
38	*E. coli*	11	R	11	R	29	S	30	S	31	S	20	S		
39	*E. coli*	20	S	11	R	33	S	ND		11	R	12	R		
40	*E. coli*	11	R	12	R	ND		29	S	23	S	20	S		
41	*E. coli*	12	R	ND		ND		31	S	21	S	21	S		
42	*K. aerogenes*	21	S	ND		33	S	35	S	25	S	11	R		
43	*E. coli*	11	R	11	R	ND		30	S	28	S	20	S		
44	*E. coli*	12	R	12	R	11	R	32	S	12	R	22	S		
45	*K. aerogenes*	10	R	8	R	33	S	35	S	30	S	20	S		
46	*E. coli*	12	R	ND		10	R	31	S	ND		21	S		
47	*E. coli*	20	S	37	S	12	R	32	S	ND		11	R		
48	*E. coli*	12	R	ND		8	R	32	S	10	R	21	S		
49	*E. coli*	12	R	ND		30	S	30	S	ND		20	S		
50	*K. aerogenes*	27	S	27	S	10	R	12	R	11	R	12	R		

S = Sensitive

R = Resistance

IZ = Inhibition zone (mm)

ND = Not detected (0.00)

5- Sensitivity test of pathogenic isolates against different tested antibiotics :

The sensitivity test of pathogenic bacterial isolates against different tested antibiotic were illustrated in table (6) and fig (3) and Plate(1,2,3) These results showed that the antibiotic nitrofurantion is more effective against isolated pathogenic bacterial organisms, which the percentage of sensitive organism reach to 68% followed by noroxin 60% septrin-30% cephracline 28%, ampicillin 24% and amikin 20%.

Table (6) : Sensitivity test of pathogenic isolates against different antibiotics

Tested antibiotics	Sensitive isolates		Resistance isolate	
	No.	%	No.	%
Nitrofurantion (F)	34	68.0	16	32.0
Amikin (AK)	10	20.0	40	80.0
Noroxin (NOR)	30	60.0	20	40.0
Septrin sulfatrimero (SXT)	15	30.0	35	70.0
Ampicilin (Am)	12	24.0	38	76.0
Cephradine (Ce)	14	28.0	36	72.0

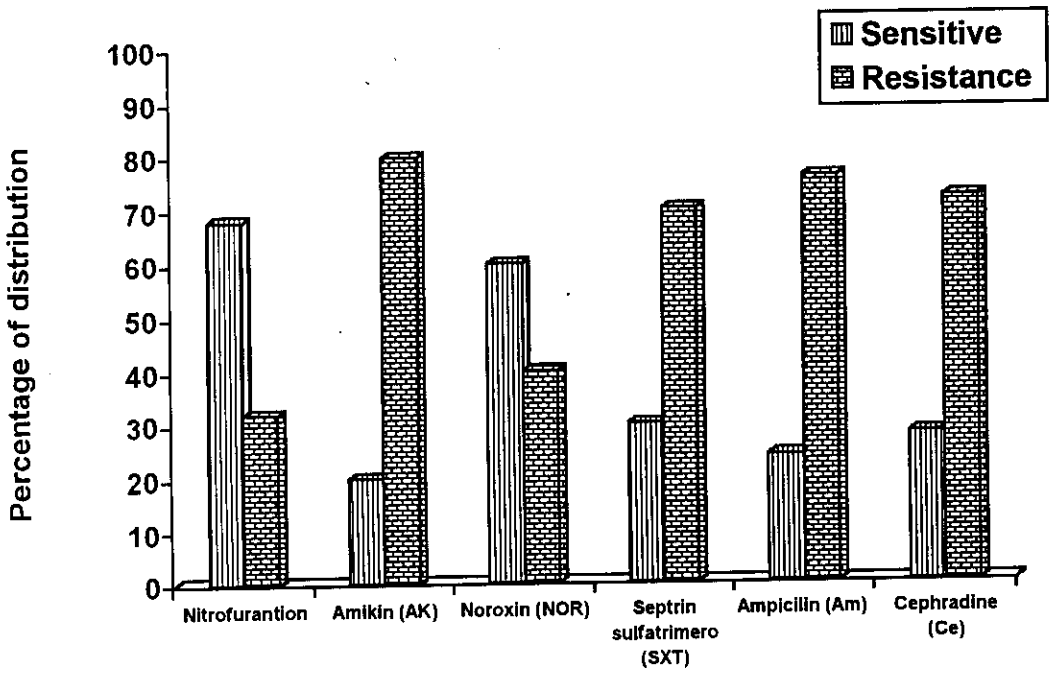


Fig. (3) : Sensitivity test of pathogenic isolates against different antibiotics.

6- Statistical analysis of sensitive and resistance pathogenic isolates against different antibiotics :

The results in table (7 a, b, c, d, e and f) showed that the sensitivity of each pathogenic bacterial isolate against one tested antibiotic to illustrate the obtained results are significant or not, all obtained results are significant and the percentage of sensitivity tested against antibiotic nitrofurantion reach to maximum value with *E. coli* (55.9%) more than *K. aerogense* (23.5%) and *P. aoroginosae*, (20.6%), the same results obtained with noroxin antibiotic. Antibiotic septrin sulfatrimero and cephradine are more affective in *K. aerogense* (60% and 50% respectively) more than *E. coli* (33.3% and 35% respectively) and *P. aeroginosae* (6.7% and 14.3 respectively).

Table (7) : Statistic analysis of sensitive and resistance pathogenic bacterial isolates against different antibiotics.

a) Antibiotic nitrofurantion

Isolated organisms	Sensitive		Resistant		X^2	P
	No.	%	No.	%		
E. coli	19	55.9	10	62.5	1.73 0.4	NS
K. aerogenes	8	23.5	5	31.3		
P. aeroginosae	7	20.6	1	6.3		

b) Antibiotic amakin

Isolated organisms	Sensitive		Resistant		X^2	P
	No.	%	No.	%		
E. coli	5	50.0	24	60.0	4.91 0.08	NS
K. aerogenes	5	50.0	8	20.0		
P. aeroginosae	0.0	0.0	8	20.0	-	

c) Antibiotic noroxin

Isolated organisms	Sensitive		Resistant		X^2	P
	No.	%	No.	%		
E. coli	17	56.7	12	60.0	0.82 0.6	NS
K. aerogenes	9	30.0	4	20.0		
P. aeroginosae	4	13.3	4	20.0		

d) Antibiotic septrin sulfatrimero

Isolated organisms	Sensitive		Resistant		X^2	P
	No.	%	No.	%		
E. coli	9	60.0	4	11.4	12.9 < 0.01*	Significant
K. aerogenes	5	33.3	24	68.6		
P. aeroginosae	1	6.7	7	20.0		

e) Antibiotic ampicillin

Isolated organisms	Sensitive		Resistant		X^2	P
	No.	%	No.	%		
E. coli	5	41.7	8	21.0	2.2 0.32	NS
K. aerogenes	5	41.7	24	63.2		
P. aeroginosae	2	16.7	6	15.8		

f) Antibiotic cephradine

Isolated organisms	Sensitive		Resistant		X^2	P
	No.	%	No.	%		
E. coli	7	50.0	6	16.7	6.01 0.04	Significant
K. aerogenes	5	35.7	24	66.7		
P. aeroginosae	2	14.3	6	16.7		

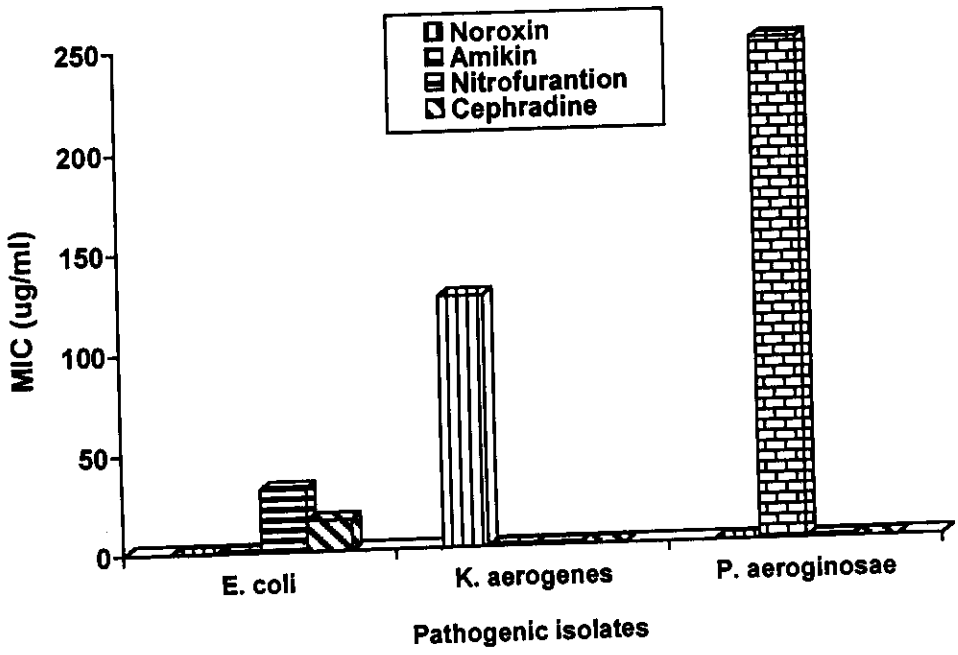
7- Minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of some antibiotics

The experimental bacteria were treated separately with different concentration of the antibiotics under test in nutrient broth and on solid agar medium and the MICs and the MBCs were determined.

The obtained data recorded in table (8) and fig. (4) revealed that the MICs of noroxin for *K. aerogenes* were 125 µg/ml, while the MBCs were 250 µg/ml, the MICs and MBCs of amikin for *P. aeroginosae* was 250 µg/ml, the MICs of nitrofuration and cephradine for *E. coli* isolated were 31.25 µg/ml and 15.625 µg/ml respectively, while MBCs of *E. coli* by nitrofurantion and cephradine were 250 and 125 µg/ml respectively.

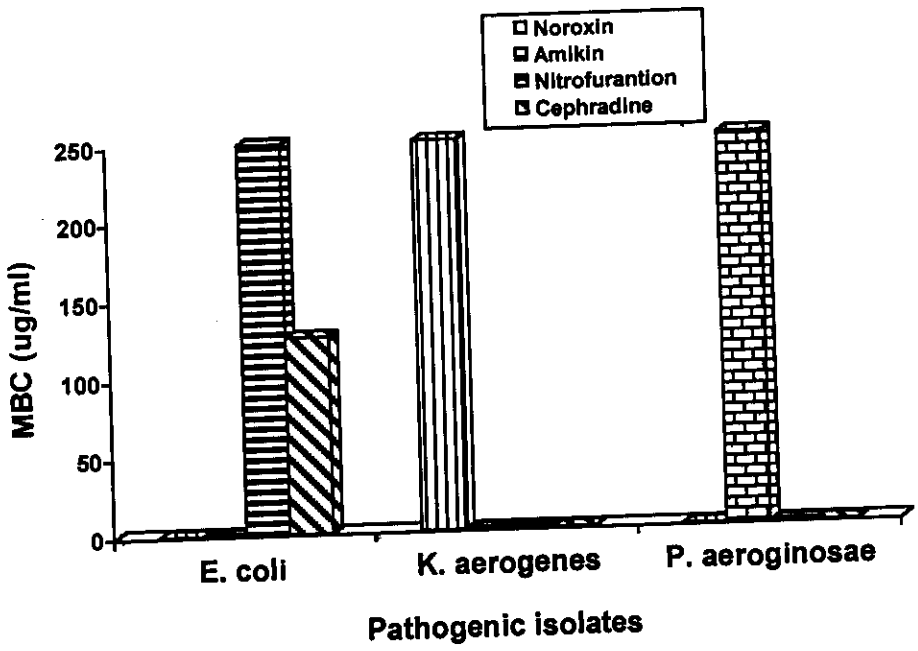
Table (8) : Minimum inhibitory concentration (MICs) (μg/ml) and minimum bactericidal concentration (MBCs) of different antibiotics.

Organism	(MIC) (μg/ml)		(MBC) (μg/ml)	
K. aerogenes	Noroxin 125		Noroxin 250	
P. aeroginosae	Amikin 250		Amikin 250	
E. coli	Nitrofuration 31.25	Cephradine 15.625	Nitrofurantion 250	Cephradine 125



(a)

Fig. (4) : a-Minimum inhibitory concentration (MICs) (μg/ml) of different tested antibiotics.



(b)

Fig. (4) : b-Minimum bacterial concentration (MBCs) (μg/ml) of different tested antibiotics.

8- The effect of different plant extracts (water and alcohol extract) on *E.coli*

The plant extracts that used in this experiment were mint, dianthus, tryme, rosemary, carawaya, habet elbaraka, against the two isolates of *E. coli* which selected previously. Data demonstrated in table (9 & 10 a, b) and fig (5,6) and plate (4) illustrated the inhibitory effect of different water and alcohol plant extracts. These results indicated that alcohol extract of mint and habet elbarka gave inhibitory effect against *E. coli* in both tested isolate if compared with water extract, which not active against both tested isolates of *E. coli.*

While, dianthus, rosemary and carawaya have effect on two selected isolates in the two cases (water and alcohol extract).

On the other *E. coli* (isolate no. 36) was more sensitive to dianthus where it recorded inhibition zone 20, 19 by using alcohol and water extract respectively and *E. coli* (isolate number 47) was more sensitive to rosemary alcohol extract where it recorded the highest inhibition zone (23 mm).

Table (9) : Diameter of inhibition zone (mm) of different plant extract (water and alcohol) on *E. coli* (isolate number 36, 47).

Plant extract	*E. coli* (isolate no 36)								*E. coli* (Isolate no 47)							
	Water				Alcohol				Water				Alcohol			
	Conc.		Diluted		Conc.		Diluted		Conc.		Diluted		Conc.		Diluted	
	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S
Mint	ND		ND		15	S	15	S	ND		ND		19	S	ND	
Dianthus	19	S	ND		2o	S	15	S	ND		ND		22	S	13	S
Thyme	15	S	ND		14	S	14	S	17	S	ND		13	S	12	S
Rosemary	N	N	ND		14	S	19	S	ND		ND		23	S	19	S
Carawya	13	S	ND		19	S	18	S	17	S	ND		22	S	15	S
Habet elbarka	ND		ND		14	S	18	S	ND		ND		14	S	14	S

Diluted = 1 : 2
S = Sensitive
R = Resistance
IZ = Inhibition zone
ND = Not detected

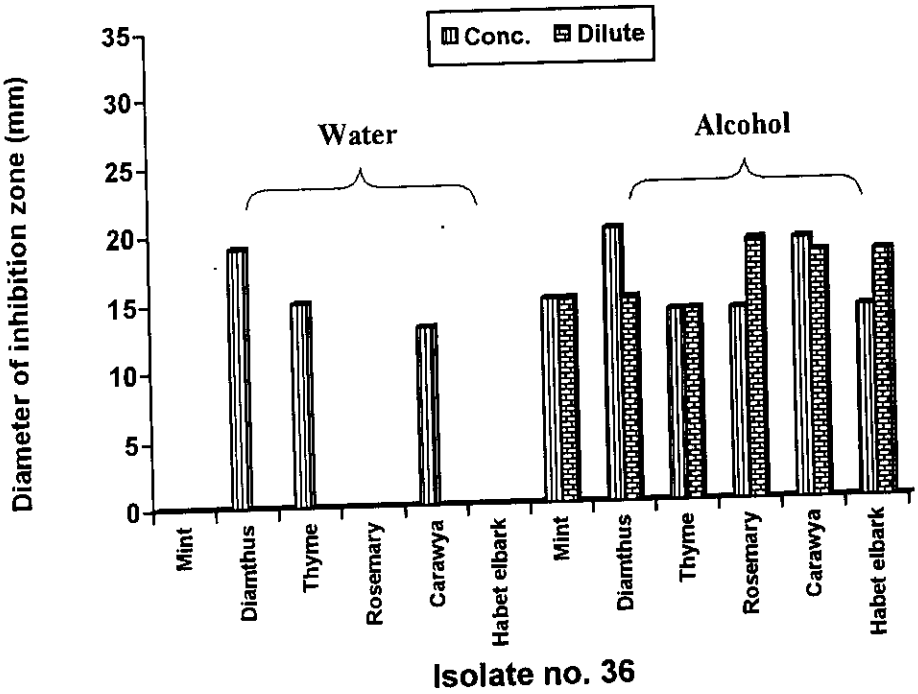


Fig. (5) : Diameter of inhibition zone (mm) of different plant extract (water and alcohol) on *E. coli* (isolate number 36).

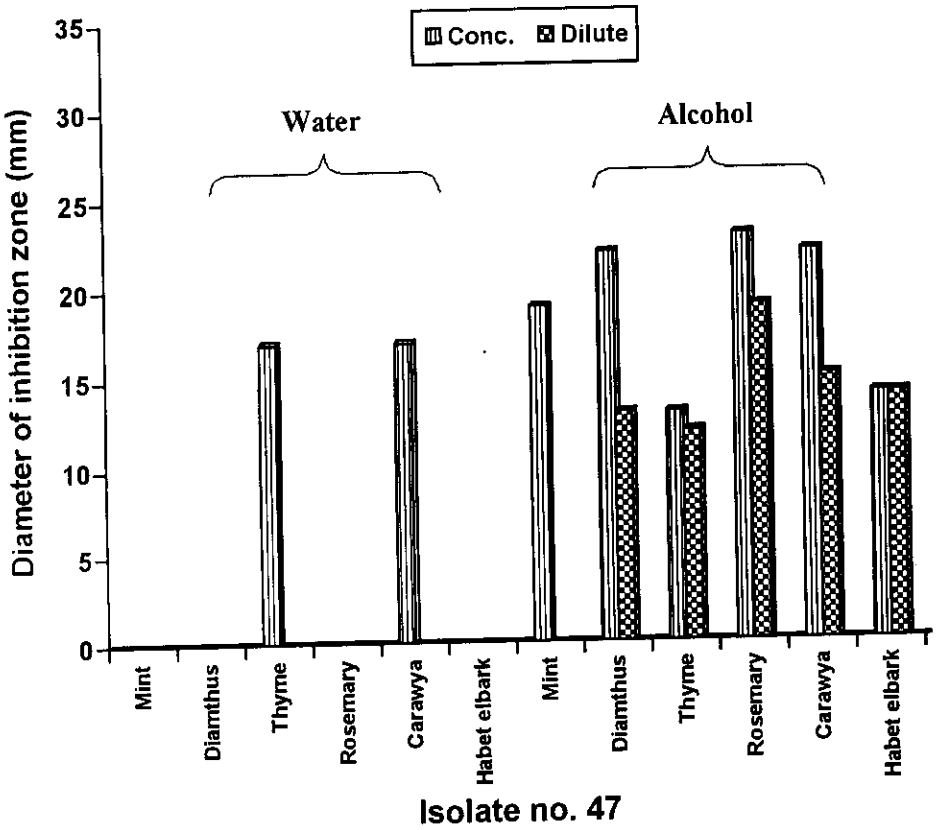


Fig. (6) : Diameter of inhibition zone (mm) of different plant extract (water and alcohol) on *E. coli* (isolate number 47).

Table (10) : Sensitivity of *E. coli* to water and alcoholic plant extracts (diluted and concentrated).

a) *E. coli* isolate no. 36

***E. coli* isolate no. 36**	Water plant extract		Alcohol plant extract	
	Conc.	Dilute	Conc.	Dilute
Sensitive	3	0	6	6
Resistant isolate	3	6	0	0

$X^2 = 17.6$ P = < 0.001 **HS (high significant)

b) *E. coli* isolate no. 47

***E. coli* isolate no. 47**	Water plant extract		Alcohol plant extract	
	Conc.	Dilute	Conc.	Dilute
Sensitive	3	0	6	5
Resistant isolate	3	6	0	1

$X^2 = 14.4$ P = < 0.002 *S (significant)

10- The effect of different plant extracts (water, alcohol) on *K. aerogenese*

The six active plant extract that used in this experiment were mint, dianthus, thyme, rosemary, carawaya, habet elbaraka. Data demonstrated in tables (11 & 12 a, b) and fig (7,8) and plate (5) illustrated the inhibitory effect of alcohol and water plant extracts on two isolates (8, 50) of *K. aerogenes*.

Alcholic extract of dianthus, thyme, mint and rosemary showed great effect against *K. aerogenes* (isolate number 50), where they recorded inhibition zones 19, 19, 21, 20 mm respectively. On the other hand water extract of dianthus, thyme and carawya give low inhibitory effect in concentrated form only in both tested isolate. The alcholic extract of dianthus and carawya gave the highest inhibition zone (21 mm) against *K. aerogenes* (isolate number 8) followed by rosemary (7 mm).

In generally, the alcoholic extracts were more effective against both tested isolates than water extract.

From the previous results we can recognize that the antimicrobial activity of dianthus, and rosemary (alcoholic extract) showed great effect against *K. aerogenes* (isolate number 8, 50).

Table (11) : Diameter of inhibition zone (mm) of different plant extract (water and alcohol) on *K. aerogenes* (isolate 8, 50).

Plant extract	*K. aerogenese* (isolate no 8)								*K . aerogenese* (isolate no 50)							
	Water				Alcohol				Water				Alcohol			
	Conc.		Diluted		Conc.		Diluted		Conc.		Diluted		Conc.		Diluted	
	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S
Mint	ND		ND		17	S	16	S	ND		ND		21	S	19	
Diamthus	18	S	ND		21	S	20	S	19	S	ND		19	S	18	
Thyme	13	3	ND		15	S	19	S	16	S	ND		19	S	18	
Rosemary	ND		ND		19	S	19	S	ND		ND		20	S	16	
Carawya	17	S	ND		21	S	ND		17	S	ND		16	S	ND	
Habet elbarka	ND		ND		17	S	17	S	ND		ND		16	S	13	

Diluted = 1 : 2

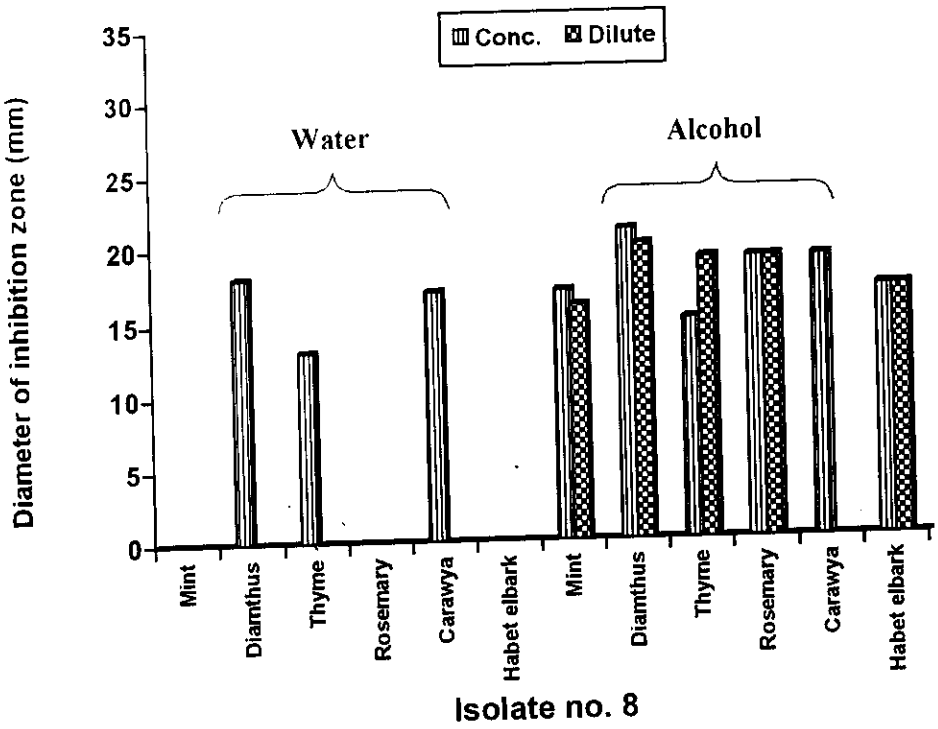


Fig. (7) : Diameter of inhibition zone (mm) of different plant extract (water and alcohol) on *K. aerogenes* (isolate 8).

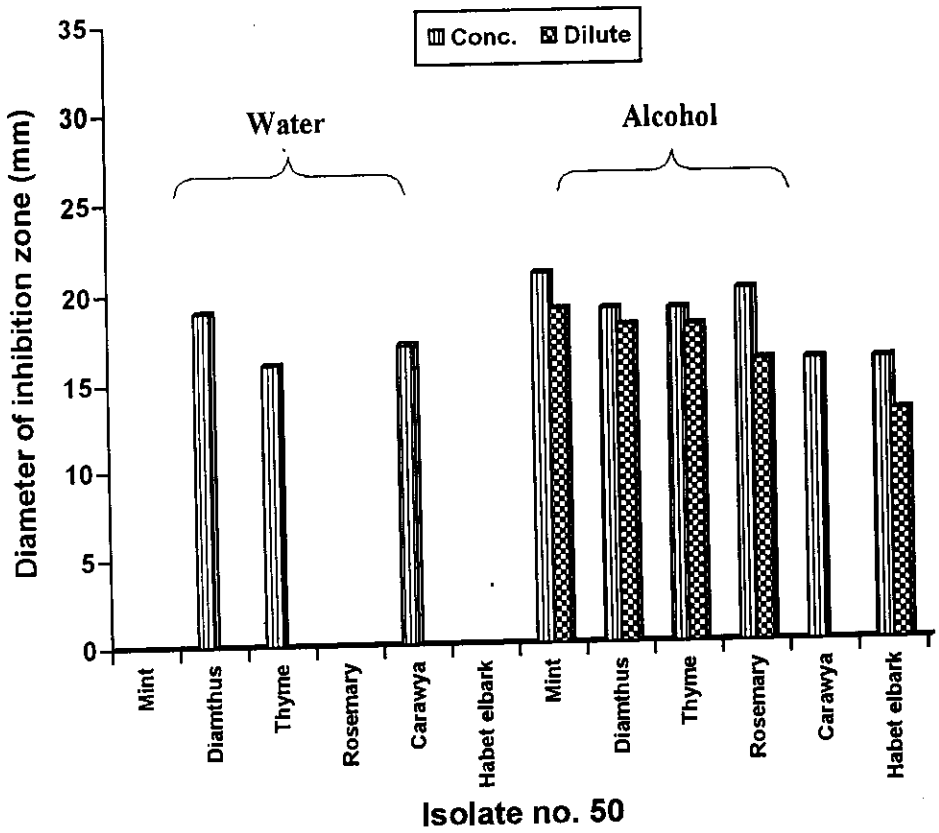


Fig. (8) : Diameter of inhibition zone (mm) of different plant extract (water and alcohol) on *K. aerogenes* (isolate 50).

Table (12) : Sensitivity of *K. aerogenese* to water and alcoholic plant extracts (diluted and concentrated).

a) *K. aerogenese* isolate no. 8

***K. aerogenese* isolate no. 8**	Water plant extract		Alcohol plant extract	
	Conc.	Diluted	Conc.	Diluted
Sensitive	3	0	6	5
Resistant isolate	3	6	0	1

$X^2 = 17.6$ P = < 0.001 **HS (high significant)

b) *K. aerogenese* isolate no. 50

***E. coli* isolate no. 47**	Water plant extract		Alcohol plant extract	
	Conc.	Dilute	Conc.	Dilute
Sensitive	3	0	6	5
Resistant isolate	3	6	0	1

$X^2 = 14.4$ P = 0.002 (significant)

11- The effect of different plant extracts (water, alcohol) against both isolates of *P. aeroginosae* (isolate no. 12 & 30)

In this experiment the same six plant extracts were used to evaluate their antimicrobial activity against *P. aeroginosae* (isolate number 12, 30). Data in tables (13 & 14 a, b) and fig (9,10) and plate(6) illustrated the inhibitory effect of the water and alcohol plant extract against *P. aeroginosae.*

The results showed that the alcoholic of different plant extracts gave highest inhibitory effect against *P. aeroginosae* (isolate number 12, 30) while water plant extract gave the lowest inhibitory effect against both tested isolates of *P. aeroginosae.*

Alcoholic extract of mint gave the highest inhibitory effect (21 mm) against *P. aeroginosae,* isolate number (30) followed by alcholic extract of dianthus and rosemary which gave 19 and 17 respectively. Alcholic extract of dianthus was the more active (17 mm) against *P. aeroginosae* isolate number (12) followed by alcoholic mint extract (16 mm), while thyme and rosemary recorded 15 mm inhibitory zone.

Table (13) : Diameter of inhibition zone (mm) of different plant extract (water and alcohol) on *P. aeroginosae* (isolate number 12, 30).

Plant extract	*P. aeroginosae* (isolate no 12)								*P. aeroginosae* (isolate no 30)							
	Water				Alcohol				Water				Alcohol			
	Conc.		Diluted		Conc.		Diluted		Conc.		Diluted		Conc.		Diluted	
	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S
Mint	ND		ND		16	S	19	S	ND		ND		21	S	17	S
Diamthus	ND		ND		17	S	17	S	ND		ND		19	S	16	S
Thyme	14	S	ND		15	S	16	S	14	S	ND		15	S	12	S
Rosemary	ND		ND		15	S	13	S	ND		ND		17	S	14	S
Carawya	13	S	ND		ND		14	S	17	S	ND		16	S	ND	
Habet elbarka	ND		ND		13	S	ND		ND		ND		16	S	14	S

Diluted = 1 : 2

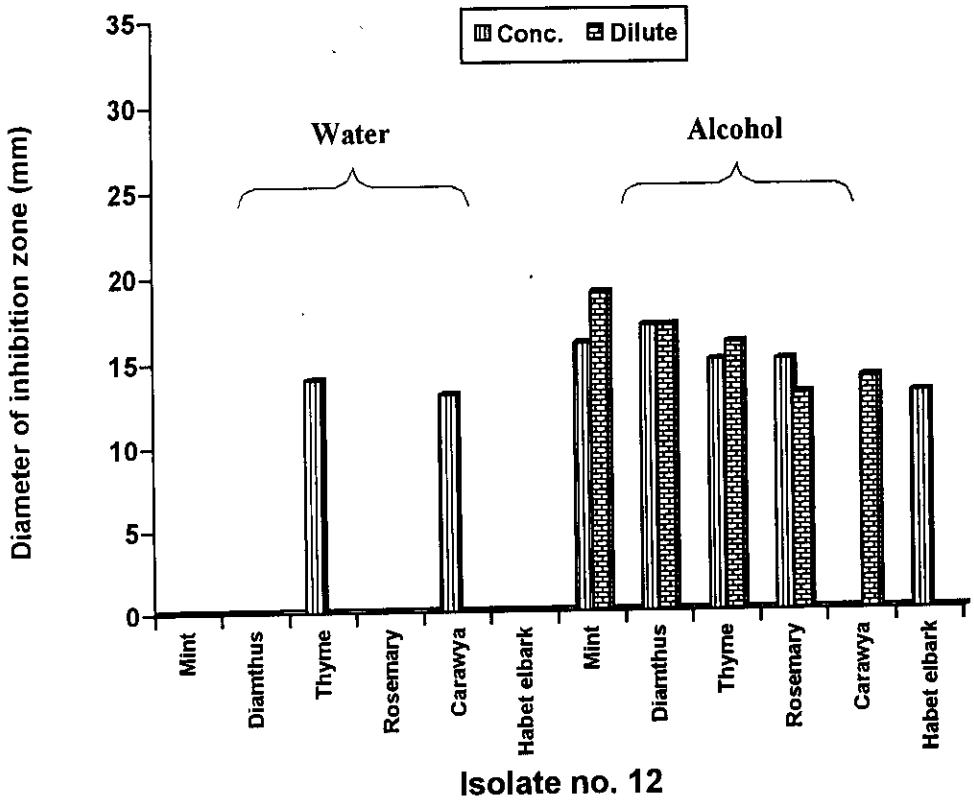


Fig. (9) : Diameter of inhibition zone (mm) of different plant extract (water and alcohol) on *P. aeroginosae* (isolate number 12).

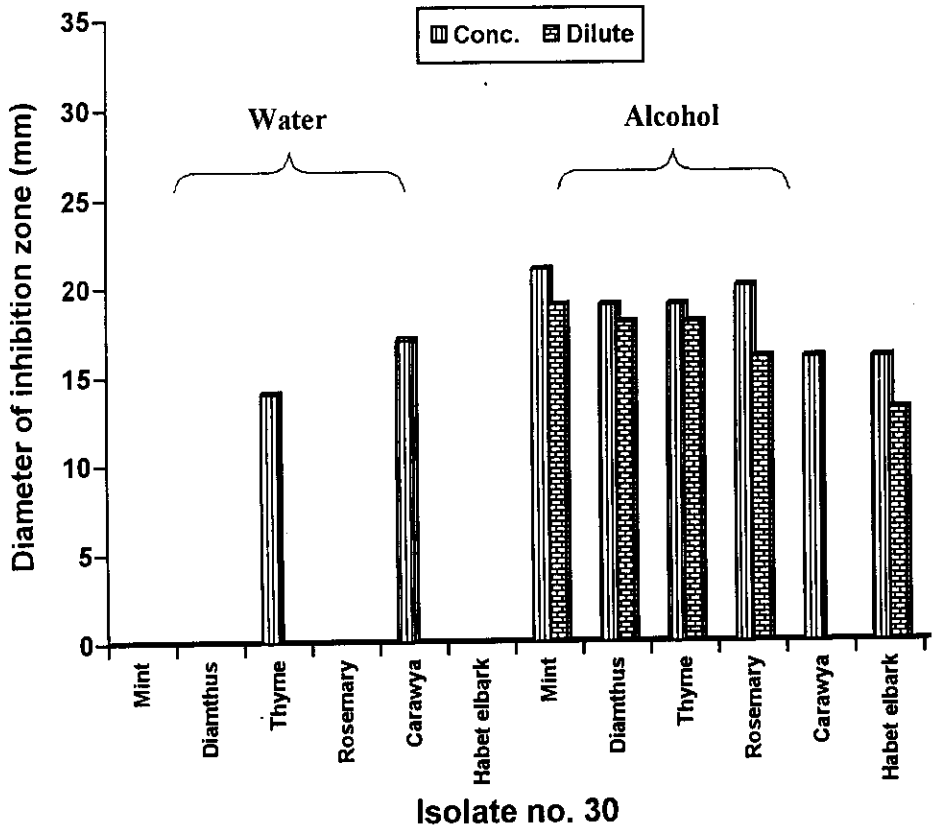


Fig. (10) : Diameter of inhibition zone (mm) of different plant extract (water and alcohol) on *P. aeroginosae* (isolate number 30).

Table (14) : Sensitivity of *P. aeroginosae* to water and alcoholic plant extracts (diluted and concentrated).

a) *P. aeroginosae* isolate no. 12

***K. aerogenese* isolate no. 12**	Water plant extract		Alcohol plant extract	
	Conc.	**Dilute**	**Conc.**	**Dilute**
Sensitive	2	0	5	5
Resistant isolate	4	6	1	1

$X^2 = 12.0$ P = < 0.01 (significant)

b) *P. aeroginosae* isolate no. 30

***K. aerogenese* isolate no 30**	Water plant extract		Alcohol plant extract	
	Conc.	**Dilute**	**Conc.**	**Dilute**
Sensitive	2	0	6	5
Resistant isolate	4	6	0	1

$X^2 = 15.27$ P = 0.0015 (significant)

12- The effect of different volatile oils (10 µl) against different isolates of *E. coli, K. aerogenes, P. aeroginosae*

In this experiment different volatile oils were used as thyme, anise, carawya, dianthus, peppermint, majoram and mint.

The concentration which used (10 µl) of these volatile oils to evaluate their antimicrobial activities against the most sensitive microorganism by using contact form (disc diffusion method).

Data in table (15) and fig(11,12,13) and plate(7) illustrated the inhibitory effect of volatile oils (10 µl) against *E. coli, K. aerogenes* and *P. aeroginosae* (the two isolates of each one).

The results showed that the inhibitory action was recorded of thyme oil against all tested organisms but the maximum value of inhibitory was recorded 40 mm with *E. coli* (isolate no. 47). While a minimum value of inhibition zone was recorded (15 mm) at the concentration (10 µl) against *P. aeroginosae* (isolate no. 30).

On the other hand, thyme oil have not inhibitory effect against *P. aeroginosae* (isolate no. 12) and *K. aerogenes* (no 8).

Anise, peppermint, majoram and mint oil (10 µl) showed a low effect (or not effect) against most isolated organisms except *E. coli* isolate number (36) which anise oil showing inhibitory (25 mm) against *E. coli* isolate number (47).

P. aeroginosae (isolate no. 12) showing resistant behavior against all tested volatile oils(10 µl) except dianthus oil showing low inhibitory action 15mm.

Table (15) : Diameter of inhibition zone (mm) of different volatile oils (10 µl) against pathogenic bacterial isolates.

Volatile oils (10 ul)	*E. coli* (isolate no 36)		*E. coli* (isolate no 47)		*P. aeroginosae* (isolate no 12)		*P. aeroginosae* (isolate no 30)		*K. aerogenes* (isolate no 50)		*K. aerogenes* (isolate no 8)	
	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S
Thyme	35	S	40	S	ND		15	S	35	S	ND	
Anise	ND		25	S	ND		ND		ND		ND	
Carawya	15	S	15	S	ND		ND		14	S	25	S
Dianthus	24	S	18	S	15	S	ND		ND		20	S
Peppermint	ND		ND		ND		ND		15	S	13	S
Majoram	ND		ND		ND		13	S	ND		ND	
Mint	ND		ND		ND		ND		ND		ND	

S = Sensitive

R = resistance

ND = not detected

IZ = Inhibition zone (mm)

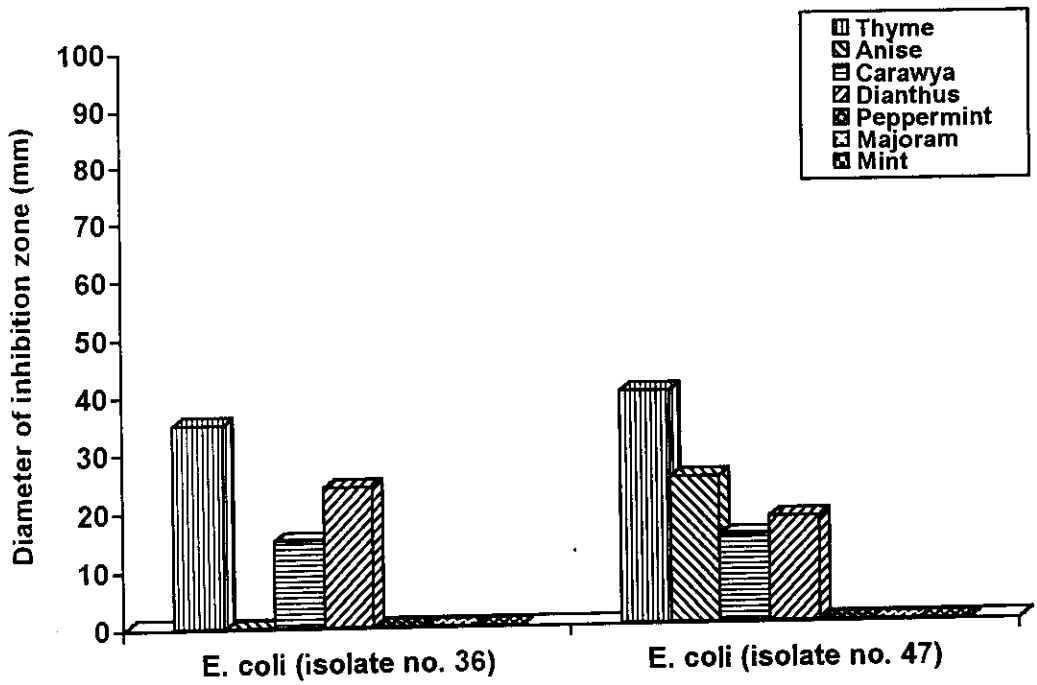


Fig. (11) : Diameter of inhibition zone (mm) of different volatile oils (10 µl) against *E.coli* isolates.

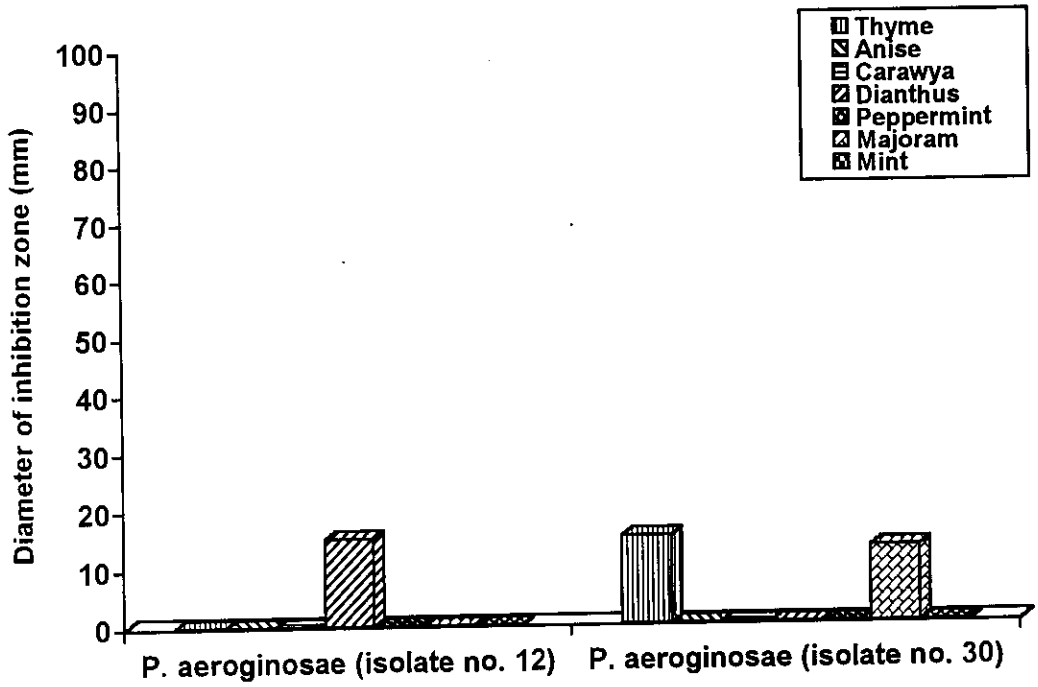


Fig. (12) : Diameter of inhibition zone (mm) of different volatile oils (10 µl) against *P. aeroginosae* isolates.

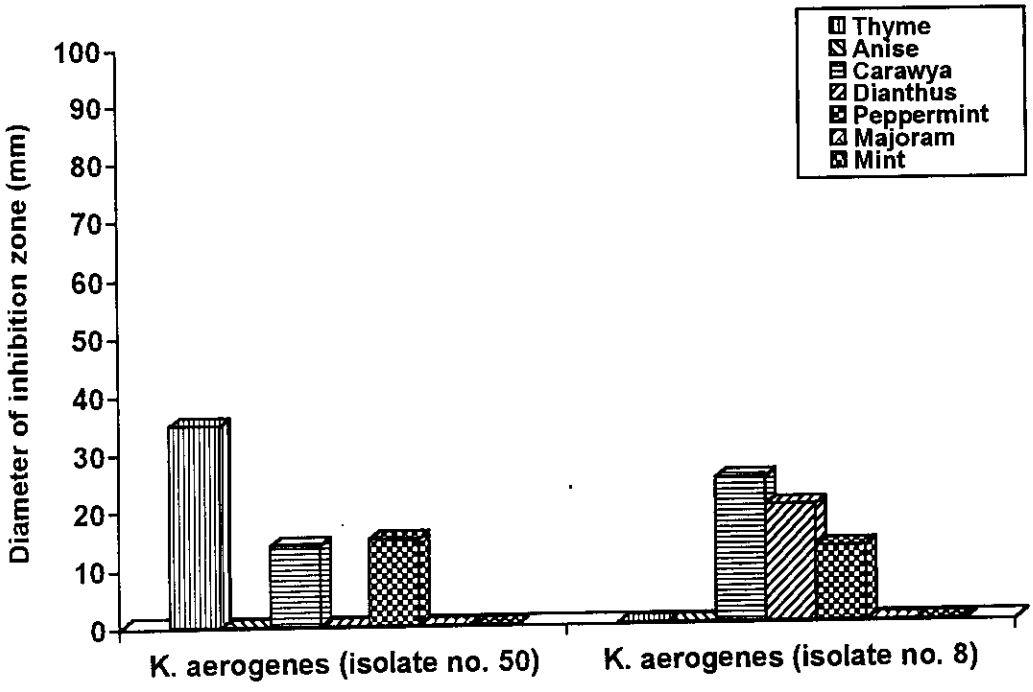


Fig. (13) : Diameter of inhibition zone (mm) of different volatile oils (10 µl) against *K. aerogenes* isolates.

13- The effect of different volatile oils (20 µl) against pathogenic bacterial isolates

The oils that used in this experiment were thyme, anise, carawya, dianthus, peppermint, majoram and mint.

The concentration which used (20 ul) of these volatile oils. Data were demonstrated in table (16)and fig (14,15,16) and plate(8). Thyme showed great effect against the most isolates especially *E. coli* where it recorded inhibition zone 28 & 50 (40 mm in the two isolate *E. coli*) while *P. aeroginosae* recorded 17 mm in (isolate no. 30) but *P. aeroginosae* (isolate no. 12) not detected any value, and we found that *K. aerogenes* (isolate no. 50) recorded 35 mm but *K. aerogenes* (isolate no. 8) not detected any value.

The effect of thyme on the isolates showed that *E. coli* isolates were more sensitive than other tested organisms to thyme.

Mint have no effect on all isolates.

Anise, peppermint, majoram and mint oil (20 µl) showed a low effect (or not effect) against most tested organisms except *E. coli* isolate number (47) which anise oil showing inhibitory action (25 mm).

P. aeroginosae (isolate no. 30) showing resistant behavior against all tested volatile oils (20 µl) except majoram oil showing low inhibitory action (14 mm)

Table (16) : Diameter of inhibition zone (mm) of different volatile oils (20 µl) against different isolates of *E. coli*, *K. aerogenes* and *P. aeroginosae*.

Volatile oils (20 ul)	*E. Coli* (isolate no 36)		*E. Coli* (isolate no 47)		*P. aeroginosae* (isolate no 12)		*P. aeraginasae* (isolate no 30)		*K. aerogenes* (isolate no 50)		*K. aerogenes* (isolate no 8)	
	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S
Thyme	28	S	50	S	27	S	17	S	35	S	35	S
Anise	55	S	55	S	ND		ND		13	S	15	S
Carawya	45	S	55	S	14	S	20	S	13	S	25	S
Dianthus	35	S	30	S	27	S	ND		20	S	24	S
Peppermint	35	S	25	S	14	S	ND		13	S	13	S
Majoram	ND		ND		ND		14	S	18	S	13	S
Mint	ND		ND		ND		ND		ND		ND	

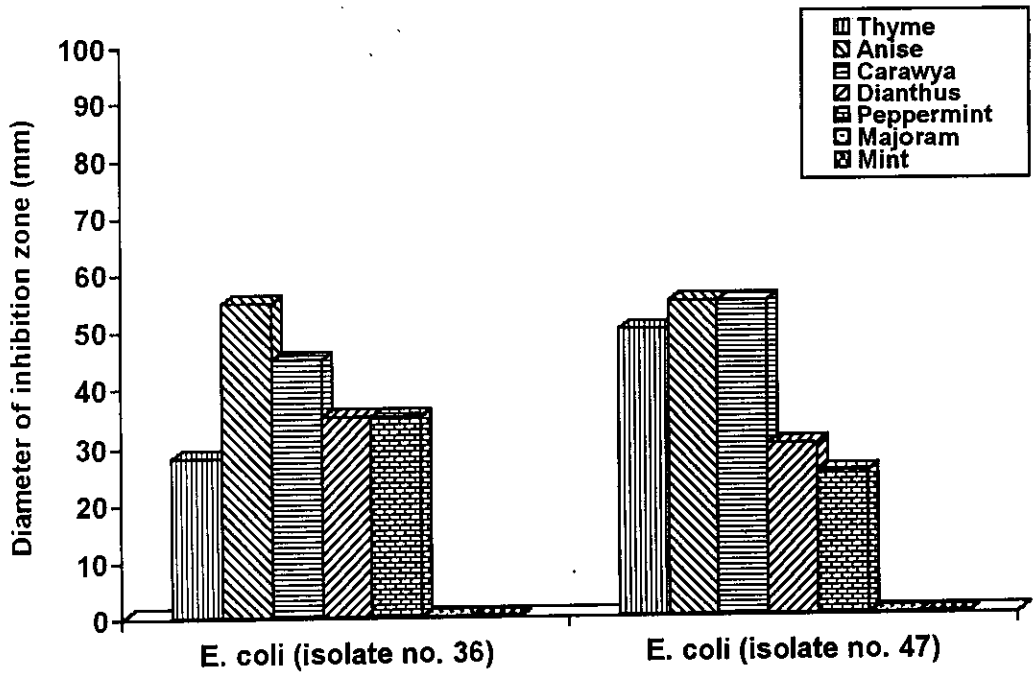


Fig. (14) : Diameter of inhibition zone (mm) of different volatile oils (20 µl) against tested isolates of *E. coli*.

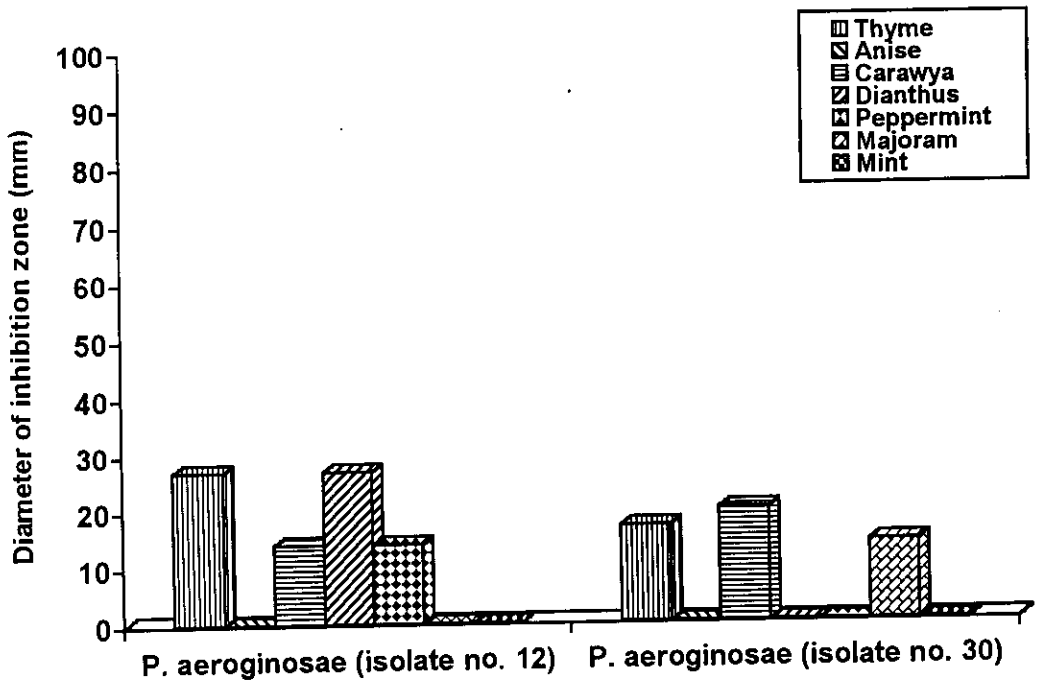


Fig. (15) : Diameter of inhibition zone (mm) of different volatile oils (20 µl) against tested isolates of *P. aeroginosae*.

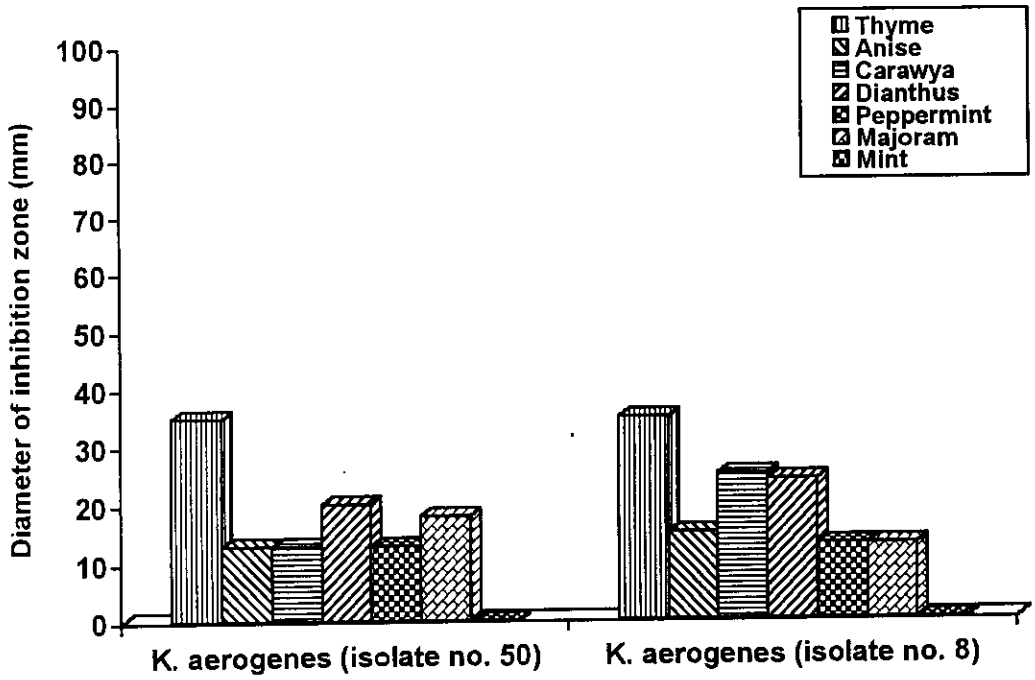


Fig. (16) : Diameter of inhibition zone (mm) of different volatile oils (20 µl) against tested isolates of *K. aerogenes*.

14- The effect of different volatile oils (30 µl) on microorganisms

In this experiment we used the same volatile oils that used in case of concentration (10 µl) and (20 µl) but in this case we used another concentration (30 µl) to evaluate their antimicrobial activities against the most sensitive microorganisms by using disc diffusion method.

Data in table (17) and fig (17,18,19)and plate(9) illustrated the inhibitory effect of these concentration of volatile oils against *E. Coli*, *P. aeroginosae* and *K. aerogenes.*

The results showed that thyme and carawya have the best effect against microorganisms and effect on all microorganisms.

Thyme recorded a maximum value of inhibition zone (50 mm) against *E. Coli* (isolate no. 47) and minimum value (17 mm) against *P. aeroginosae* (isolate no. 30).

Anise, peppermint, majoram and mint oil (30 µl) showed a low effect (or not effect) against most tested organisms except *E. Coli* (the two isolate) which anise oil showing inhibitory action (55 mm).

P. aeroginosae (isolate no. 30) showing resistant behavior against all tested volatile oils (30 ul) except carawya oil showing low inhibitory action (20).

Table (17) : Diameter of inhibition zone (mm) of different volatile oils (30 µl) against different isolates of *E. coli*, *K. aerogenes* and *P. aeroginosae*.

Volatile oils (30 ul)	*E. coli* (isolate no 36)		*E. coli* (isolate no 47)		*P. aeroginosae* (isolate no 12)		*P. aeroginosae* (isolate no 30)		*K. aerogenes* (sample no 50)		*K. aerogenes* (isolate no 8)	
	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S	IZ	S
Thyme	28	S	50	S	27	S	17	S	35	S	35	S
Anise	55	S	55	S	ND		ND		15	S	15	S
Carawya	45	S	55	S	14	S	20	S	22	S	15	S
Dianthus	35	S	30	S	27	S	ND		20	S	24	S
Peppermint	35	S	25	S	18	S	ND		13	S	13	S
Majoram	ND		ND		ND		ND		18	S	13	S
Mint	ND		ND		ND		ND		ND		ND	

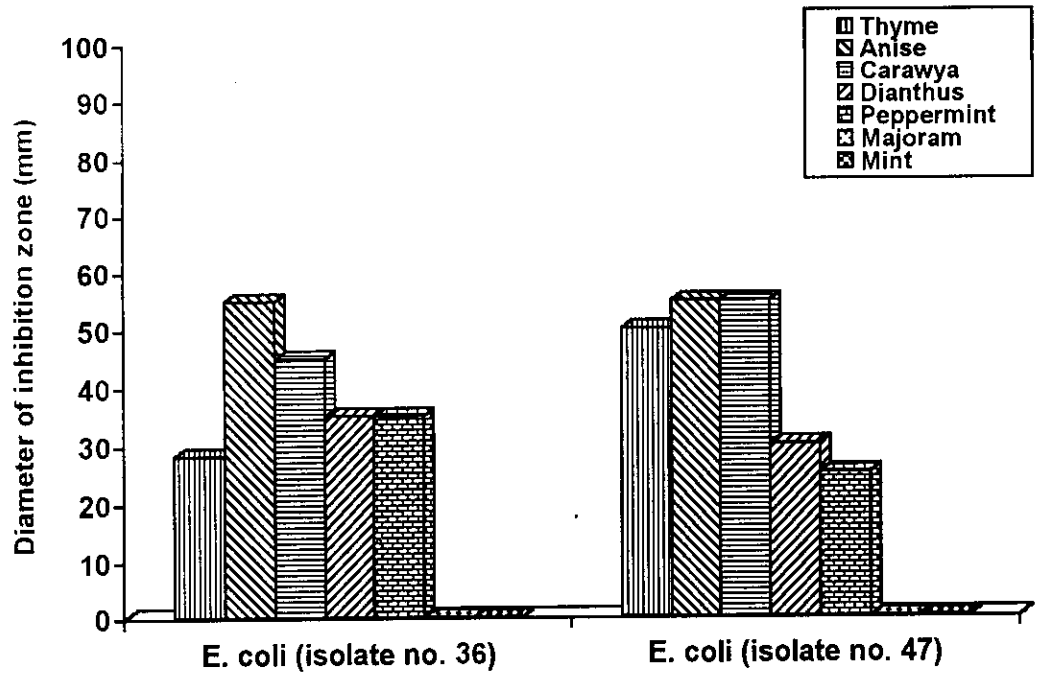


Fig. (17) : Diameter of inhibition zone (mm) of different volatile oils (30 µl) against tested isolates of *E. coli.*

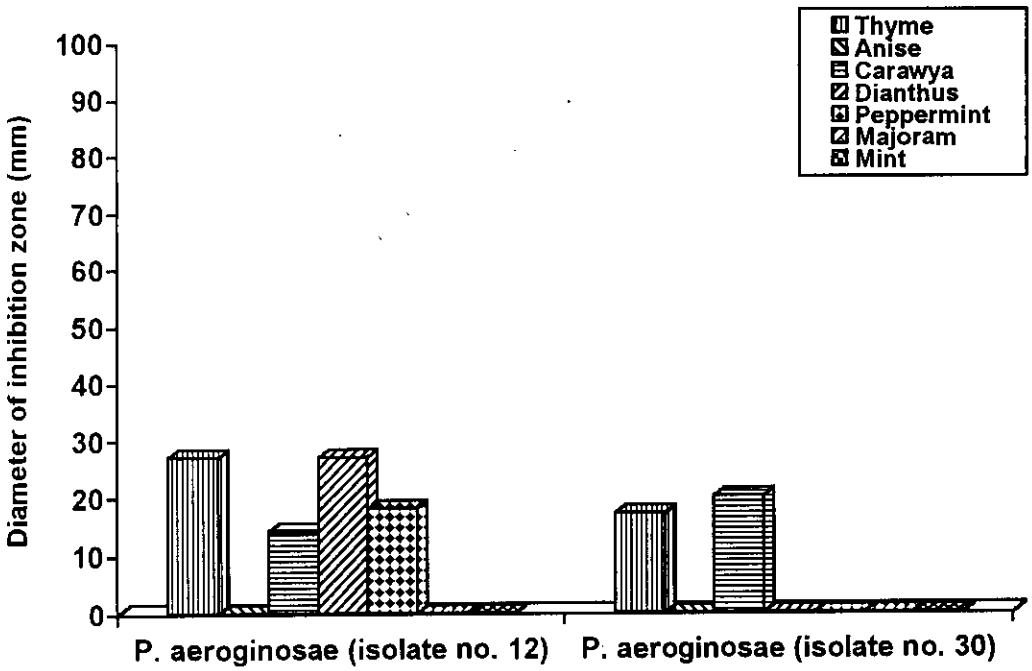


Fig. (18) : Diameter of inhibition zone (mm) of different volatile oils (30 µl) against tested isolates of *P. aeroginosae.*

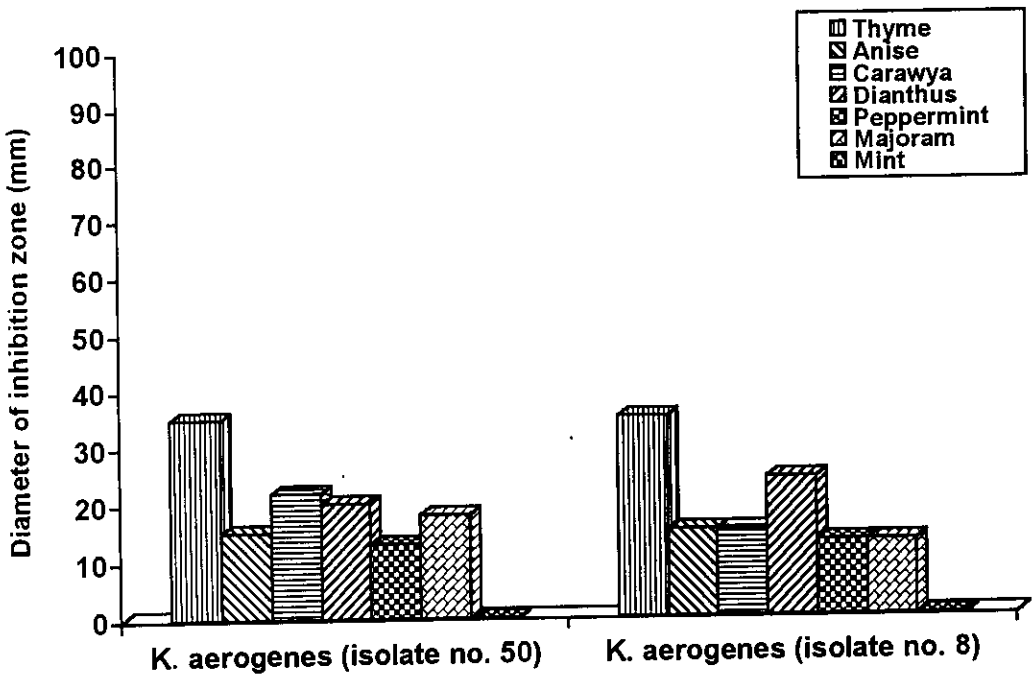


Fig. (19) : Diameter of inhibition zone (mm) of different volatile oils (30 µl) against isolate of *K. aerogenes*.