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Response of pathogenic bacteria isolated from wound infections to Antibiotics and some Essential oils from surgery department at Benha university hospital

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Abstract

A total of 53 specimens were obtained from patients suffering from wound infections in surgery department at Benha university hospital in Qaliubiya province. The bacterial isolates identified as *citrobacter freundii* (41.02%), *staph aureus* (23.07%), *pseudomonas aeruginosa* (17.9%), *coagulase negative staph* (12.8%) and *Klebsiella pneumonia* (5.12%).

The most effective antibiotics were ciprofloxacin, Amikacin and Gentamycin against all gram negative bacteria, in case of gram positive bacteria, the most effective antibiotics were Vancomycin against *staph aureus* and Clindamycin against *coagulase negative staph.* Beta lactam (Ampicillin, Amoxicillin clavulanate and cefoxitin) were the most resistant antibiotics against Gram negative and Gram positive bacteria.

Essential oils have more antimicrobial effect against gram positive bacteria than gram negative bacteria.

Combination between antibiotics and essential oils were more effective against different bacterial isolates than using both individually.

All beta lactamase producing strains of *Klebsiella pneumonia* and *co agulase negative staph* (100%), (87.5%) of *Citrobacter freundii*, (80%) of *pseudomonas aeruginosa* and (85.7%) of *staph aureus* gave positive result by the iodometric method.

1 - INTRODUCTION

Wound infection occurs as a result of the disruption of skin membrane and subsequent contamination or colonization by Microorganisms. It can be caused either by trauma (laceration road traffic or burns) or surgical operational procedures or medical incision that could result in open or closed wound infections **[1].**

Organisms commonly found in infected wounds include Gram positive cocci such as *Staph aureus*, *Streptococcus species*, Gram negative bacilli mostly *Acinetobacter*, *Enterobacter*, *Escherichia coli*, *Proteus spp.*, *Pseudomonas Aeruginosa* and anaerobic bacteria such as *Propionibacterium spp*. and *Klebsiella spp*. **[2].**

IN order to fight bacterial infections, medicine has largely relied on antibiotics, which are naturally occurring or artificially created chemical substances capable of killing or controlling the growth of bacteria. In present times, however, bacteria have developed resistance to many of the antibiotics commonly used to treat infections **[3].**

In light of the recent emergence of bacteria which are resistant to multiple antimicrobial drugs, posing a challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such microorganisms becomes patent**[4].** Different essential oils have been reported to possess antibacterial activities against gram positive bacteria including *Staphylococcus aureus, Bacillus species* and gram negative bacteria namely *E. coli, Salmonella anteritidis, Shigella flexneri,* and pathogenic fungus specifically *Candida albicans* **[5].**

The persistence of antibiotic resistance urges the need of finding new therapies against the multi drug resistant bacteria. Combination therapy combining conventional antibiotics and essential oils is currently blooming and represents a potential area for future investigations. This new generation of phytopharmaceuticals may shed light on the development of new pharmacological regimes in combating antibiotic resistance **[6].**

After the extensive usage of penicillins and cephalosporins, bacteria have developed resistance via different mechanisms. β- Lactamase enzyme production is the first developed and the most important mechanism of resistance to β-lactams **[7]**. Over the years several methods viz. acidometric method, iodometric method, chromogenic cephalosporin method **[7]** and microbiological method **[8]** have been developed to detect â-lactamase production of bacteria.

2- MATERIALS AND METHODS

-Collection of samples

The specimens were randomly collected on sterile cotton swabs from infected wounds in surgery department at Benha University hospital then transferred to laboratory of microbiology and immunology in Medical Microbiology and Immunology Department, Faculty of Human Medicine, Benha University, Egypt.

- Isolation and Identification of bacteria

Swabs collected were streaked on blood agar and MaCconkey agar media then incubated aerobically at 37℃ for 24hrs. Gram stain, physiological and biochemical tests were performed on colonies from primary cultures for identification of the isolates according to Bergey's manual **[9].**

- Preparation of the bacterial suspension

The inoculum was prepared by picking 5-10 colonies of each isolate with a sterile wire loop and suspended in into 2.5ml of sterile distilled water. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 Barium sulphate solution. Suspension was taken by a sterile cotton swab then streaked the surface of all the plate in three different planes.

- Antimicrobial sensitivity of antibiotics against Gram negative and Gram positive bacterial isolates

Antibiotic used in sensitivity patterns of isolated bacteria suspected to be Gram Negative bacteria and Gram positive bacterial isolates illustrated in table (1, 2).Susceptibility of the bacterial isolates to antibiotic discs (Con. µg/disc) was performed by Kirby-Bauer disk diffusion technique according to criteria set by CLSI 2015 **[10].**

Table (1): Antibiotics used in antimicrobial sensitivity of antibiotics against Gram negative bacterial isolates

Antibiotic class	Antibiotics	Concentration (Mg/disk)
Penicillin	Ampicillin(Amp)	10Mg
Amino glycosides	Gentamycin(CN)	10Mg
ß Lactam	Amoxicillin clavulanate(AMC)	30Mg
Amino glycosides	Amikacin(AK)	30Mg
Fluoroquiolones	Ciprofloxacin (CIP)	5Mg

Table (2): Antibiotics used in antimicrobial sensitivity of antibiotics against Gram positive bacterial isolates

Antibiotic class	**Antibiotics**	**Concentration (Mg/disk)**
Cephalosporin	Cefoxitin(fox)	30 Mg
Lincosamides	Clindamycin(DA)	2 Mg
Macrolides	Azithromycin (Azm)	15 Mg
Amino glycosides	Gentamycin(CN)	10 Mg
Glycopeptides	Vancomycin(va)	30 Mg

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Antimicrobial activity of the Essential oils against Gram negative and Gram positive bacterial isolates

Types of Essential oils tested on bacterial isolates were purchased from local super markets and stored in full dark vials at 4 ºC. Oils used illustrated in table (3).The antimicrobial activity of the Essential oils against Gram positive and gram negative bacterial isolates was assayed by using agar wells diffusion method. Wells of 6 mm in diameter

were made using a sterile cork borer in solidified agar and 50 µ l of the test oils were added to the wells. Plates were left for one hour at 4ºC and then incubated for 24 hrs at 37ºC. Wells without oils were considered as controls **[11].** Inhibition zones were measured in mm and classified as sensitive, intermediate and resistant according to the standardized table supplied by approved NCCLS 2012**[12]** for any antimicrobial agent.

Table (3): Essential oils used in antimicrobial sensitivity of essential oils against Gram negative and Gram positive bacterial isolates

Family name	Scientific (Latin)name	English name	Arabic name
Lamiaceae	Ocimum basilicum	Basil	الريحان
Ranunculaceae	Nigella sativa L.	Black seed	حبة البركة
Lamiaceae	Rosmarinus officinalis L.	Rose marry	حصا اللبان
Myrtaceae	Syzyglum romaticum L.	Clove	القرنفل
Lamiaceae	Thymus vulgaris	Thyme	الزعتر

- Effect of combination between antibiotics and essential oils againstGram negative and Gram positive bacterial isolates

The antimicrobial activity of five commercial essential oils in combination with antibiotics was performed by using Disk diffusion test (indirect contact of essential oils)**[13].** By using the sterile forceps, the disks of selected antibiotics impregnated with 50ul of oils were placed on the inoculated plates and then incubated at 37 ºC for 18-24 hrs .Inhibition zones were measured in mm and classified as sensitive, intermediate and resistant according to the standardized table supplied by approved NCCLS 2012 **[12]** for any anti microbial agent.

- Detection of beta lactamase by tube Iodometric method

The test is done by preparing a numbers of sterilized test tubes (blank, sample (n)).Added 100µl of penicillin G reagent in sample test tube (n) and blank tube. The tested bacteria were removed with a large inoculating loop from an 18 -24 hrs nutrient agar culture and inoculated in to test tube(n), blank tube leave (as control) without inoculated. Incubated the tubes at 37°c for 30 min. Added 50 µl of freshly starch solution then mixed all the tubes. Added 20µl of Iodine solution, a blue color immediately developed due to reaction of the iodine with the starch. The reaction mixture was further rotated for up to 1 min. Rapid decolorization occurred if the penicillin was hydrolyzed ; such appositive reaction indicated β lactamase activity .persistence of the blue color for longer than10 min constituted a negative test and indicated that the penicillin molecules had not under gone β lactam ring cleavage.

3 - RESULTS AND DISCUSSION

- Isolation and Identification of bacterial isolates

A total 53 wound swab, 36 wound swabs (67. 9%) were positive in bacterial growth ((91.7%) of culture positive wounds showed mono-microbial growth and (8. 3%) showed mixed growth) and only17 samples (32. 1%) were negative in bacterial growth **(table4)**, the overall bacterial isolation rate of 67.9% is comparable with the rate reported similar studies in Ethiopia **[14]** and Cameroon **[15].** Similarly high percentage of mono-microbial growth was reported in India (86-100%) and Pakistan (98%) **[16, 17]**

Table (4): patterns of growth in wounds (%)

Wound type	Growth						No growth		Total		P value
	Single growth		Mixed growth		Total						
	No	%	No	%	No	%	No	%	No	%	
Diabetic foot ulcer	10	90.9	1	9.1	11	84.6	2	15.3	13	24.5	**>0.05**
Post operative	9	81.8	2	18.2	11	55	9	45	20	37.7	
Abscess	9	100	-	-	9	69.2	4	30.8	13	24.5	
Burns	3	100	-	-	3	60	2	40	5	9.4	
Septic wound	2	100	-	-	2	100	-	-	2	3.7	
Total	**33**	**91.7**	**3**	**8.3**	**36**	**67.9**	**17**	**32.1**	**53**	**100**	

FET = 3.27 p = 0.917 (p value > 0.05, is non significant**)**

Citrobacter freundii(41.02%) and *Staphylococcus aureus* (23.07%)were the most common, followed by *Pseudomonas aeruginosa* (17.9%), *Coagulase negative Staphylococcus* (12.8%), *Klebsiella spp*.(5.12%) Table (5). This finding is consistent with other studies **[18, 19].**

Table (5) Percentage occurrence of bacterial isolates in wounds

Isolates	Total no. Isolated	% of isolated
Klebsiella pneumonia	2	5.12
Citrobacter freundii	16	41.02
Pseudomonas aeruginosa	7	17.9
Staph aureus	9	23.07
Coagulase negative staph	5	12.8
Total	**39**	**100**

- Antimicrobial sensitivity of Antibiotics against Gram Negative and Gram positive bacterial isolates

Gram negative bacterial isolates show highest resistance to Ampicillin and amoxicillin clavulanate,(71.42%)of *pseudomonas aeruginosa* resistant to two antibiotics; *citrobacter freundii*((100%) resistant to Ampicillin ,(68. 75%)resistant to amoxicillin clavulanate);*klebsiella pneumonia*(100%) resistant to two antibiotics. among gram positive isolates, *Staphylococcus aureus* showed highest resistance to cefoxitin(77. 8%) and Gentamycin (66. 7%).(table 6 , fig.1)and(table7, fig. 2). Resistance to such most commonly used antimicrobial agents has been reported by many authors **[14, 20, 21]**

Table (6): Antimicrobial sensitivity of antibiotics against Gram negative bacterial isolates by Disk diffusion method.

Antibiotics	*Klebsiella pneumonia*			*Citrobacter freundii*			*Pseudomonas aeruginosa*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin (10 Mg / disk)	-	-	2(100)	-	-	16(100)	2(28.6)	-	5(71.42)
Gentamycin (10 Mg / disk)	1(50)	-	1(50)	13(81.25)	-	3(18.75)	7(100)	-	-
Amoxicillin-clavulanate (30 Mg / disk)	-	-	2(100)	5(31.25)	-	11(68.75)	2(28.6)	-	5(71.42)
Amikacin (30 Mg / disk)	1(50)	-	1(50)	14(87.5)	-	2(12.5)	6(85.7)	1(14.3)	-
Ciprofloxacin (5 Mg / disk)	1(50)	-	1(50)	11(68.75)	-	5(31.25)	6(85.7)	1(14.3)	-

Total number of *Klebsiella pneumonia* (2), *Citrobacterfreundii*(16), *Pseudomonas aeruginosa* (7)

Key: S = sensitive; R= Resistant; I= intermediate

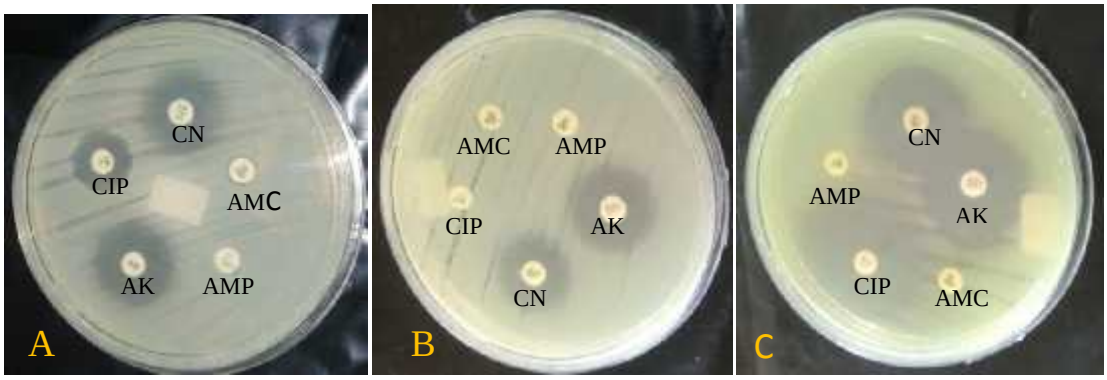

Fig (1) Photograph of antimicrobial sensitivity of antibiotics against Gram negative bacterial isolates (Disk diffusion method)

Amp (Ampicillin), CN (Gentamycin), AMC (Amoxicillin Clavulanate), AK (Amikacin), CIP (Ciprofloxacin)**.**

A) Photograph of antimicrobial sensitivity patterns of antibiotics against *Klebsiella pneumonia*, (B) Photograph of antimicrobial sensitivity patterns of antibiotics against *Citrobacter freundii*, (c) Photograph of antimicrobial sensitivity patterns of antibiotics against *Pseudomonas aeruginosa*

Table (7): **Antimicrobial sensitivity of antibiotics against Gram positive bacterial isolates by disk diffusion method**.

Antibiotics	*Staph aureus*			*Co agulase negative staph*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Cefoxitin(30mg/disk)	2(22.22)	-	7(77.8)	1(20)	-	4(80)
Clindamycin(2mg/disk)	5(55.55)	-	4(44.44)	4(80)	-	1(20)
Azithromycin(15mg/disk)	5(55.55)	-	4(44.44)	3(60)	-	2(40)
Vancomycin(30 mg / disk)	8(88.9)	-	1(11.11)	2(40)	-	3(60)
Gentamycin(10 mg / disk)	3(33.33)	-	6(66.7)	2(40)	-	3(60)

Total number of *staph aureus* (9), *coagulase negative staph* (5)

Key: S = sensitive; R = Resistant; I intermediate

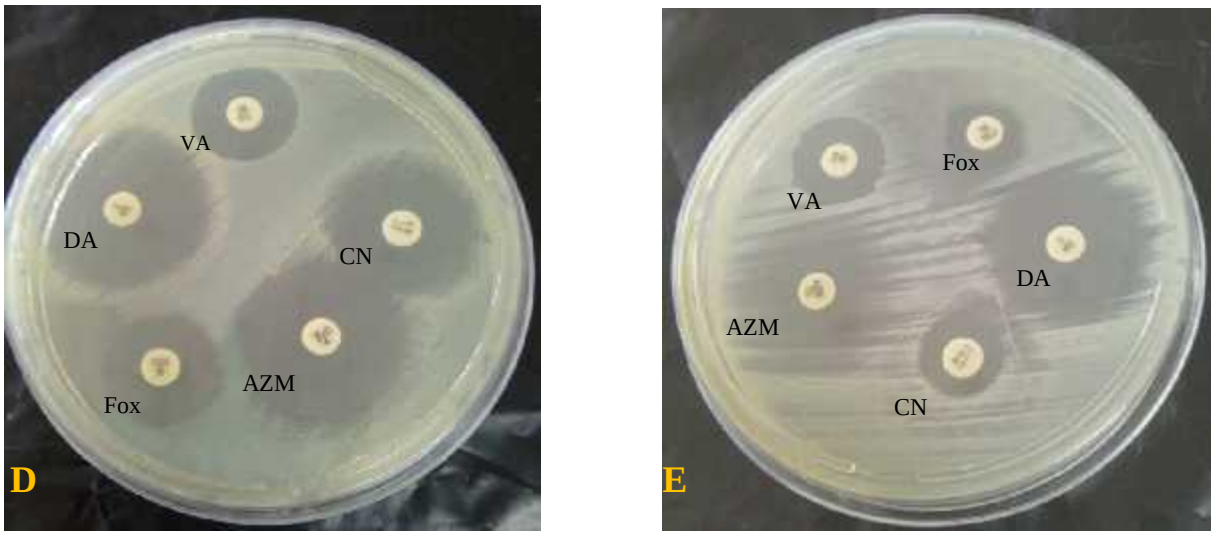

Fig (2): Photograph of antimicrobial sensitivity of antibiotics against Gram positive bacterial isolates (Disk diffusion method)

Fox (cefoxitin), Clindamycin (DA), Azm (Azithromycin*)*, CN *(*Gentamycin), VA (vancomycin*)*

(D)Photograph of antimicrobial sensitivity of antibiotics against *staph aureus*, (E) Photograph of antimicrobial sensitivity of antibiotics against *coagulase negative staph*

- Antimicrobial activity of the Essential oils against Gram negative and Gram positive bacterial isolates

This study has shown that all gram negative bacterial isolates resistant to essential oils, only one strain of *pseudomonas* show sensitivity to essential oils (table 8and fig. 3).Many researches work confirm that Gram negative bacteria are more resistant against essential oils because of the cell wall structure. Gram-negative bacteria have an outer lipopolysaccharide wall that can work as a barrier against toxic agents **[22].**

The recent study of these oils was found to be more effective on Gram positive than Gram negative bacteria (table 9and fig. 4), It may be due to absence of lipopolysaccharide layer in gram positive bacteria that may have acted as a barrier against any incoming bimolecular, this agree with **[23,24,25].**

Table (8): Antimicrobial sensitivity of Essential oils against Gram negative bacterial isolates by well diffusion method.

Essential oils	*Klebsiella pneumonia*			*Citrobacter freundii*			*Pseudomonas aeruginosa*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Basil	-	-	2(100)	-	-	16(100)	1(14.3)	-	6(85.7)
Black seed	-	-	2(100)	-	-	16(100)	1(14.3)	-	6(85.7)
Rose marry	-	-	2(100)	-	-	16(100)	1(14.3)	-	6(85.7)
Clove	-	-	2(100)	-	-	16(100)	-	-	7(100)
Thyme	-	-	2(100)	-	-	16(100)	-	-	7(100)

Total number of *Klebsiella pneumonia* (2), *Citrobacter freundii* (16), *Pseudomonas aeruginosa* (7)

Key: S = sensitive; R= Resistant; I= intermediate

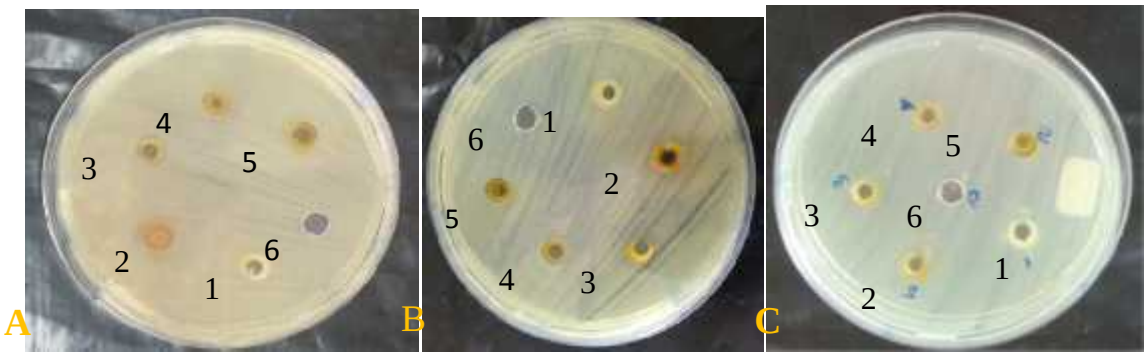

Fig (3): Photograph of antimicrobial sensitivity of essential oils against Gram negative bacterial isolates (well diffusion method)

1 =Basil oil, 2 =black seed oil, 3 =Rose marry oil, 4 = clove oil, 5 = thyme oil, 6= control (without oil)

(A) Photograph of antimicrobial sensitivity of essential oils against *Klebsiella pneumonia*, (B) Photograph of antimicrobial sensitivity of essential oils against *Citrobacter freundii*, (c) Photograph of antimicrobial sensitivity of essential oils against *Pseudomonas aeruginosa*

Table (9): Antimicrobial sensitivity of Essential oils against Gram positive bacterial isolates by well diffusion method.

Essential oils	*Staph aureus*			*Co agulase negative staph*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Basil	5(55.55)	-	4(44.44)	1(20)	-	4(80)
Black seed	9(100)	-	-	4(80)	-	1(20)
Rose marry	8(88.9)	1(11.11)	-	4(80)	-	1(20)
Clove	1(11.11)	-	8(88.9)	-	-	5(100)
Thyme	-	-	9(100)	-	-	5(100)

Total number of *staph aureus* (9), *coagulase negative staph* (5)

Key: S = sensitive; R= Resistant; I =intermediate

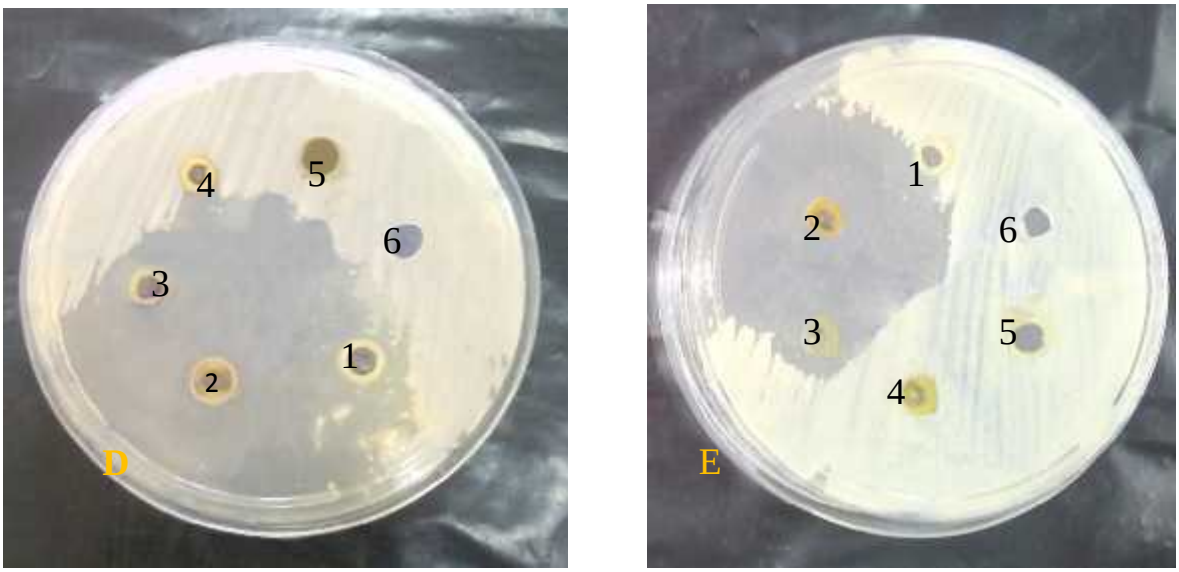

Fig (4): Photograph of antimicrobial sensitivity of essential oils on Gram positive bacterial isolates (well diffusion method)

1= Basil oil, 2 =black seed oil, 3=Rose marry oil, 4=clove oil, 5=thyme oil, 6=control (without oil)

(D) Photograph of antimicrobial sensitivity of essential oils against *staph aureus*, (E) Photograph of antimicrobial sensitivity of essential oils against *co agulase negative staph*

- Effect of combination between antibiotics and essential oils againstGram negative and Gram positive bacterial isolates .

The present study was extended to include the combination between essential oils (black seed oil, basil oil, rosemary oil, thyme oil, clove oil) and antibiotics (Ampicillin, Gentamycin, Amoxicillin clavulanate, Amikacin, Ciprofloxacin) against gram negative bacteria and antibiotics (Cefoxitin, Clindamycin, Azithromycin, Vancomycin, Gentamycin) against gram positive bacteria. The results show that combination between these antibiotics and essential oils gave synergistic effect against different isolates more than each individual alone (table10, fig. 5to table 19, fig.14) against gram negative and gram positive bacteria. These agree with Mostafa 2005 **[26]** who reported that the combination between oils and antibiotics was more active than each individual alone**.** Rosato et al. 2007 **[27]** reported that combination between essential oils and antibiotics in the treatment of infection caused by bacterial species was likely to reduce the side effects of antibiotics.

Table (10): Effect of combination between basil oil and antibiotics against Gram negative bacterial isolates by disk diffusion method

Pair combinations	*Klebsiella pneumonia*			*Citrobacter freundii*			*Pseudomonas aeruginosa*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin/ basil oil	-	-	2(100)	1(6.25)	-	15(93.75)	-	-	7(100)
Gentamycin/ basil oil	1(50)	-	1(50)	-	6(37.5)	10(62.5)	3(42.8)	1(14.3)	3(42.8)
Amoxicillin-clavulanate/ basil oil	-	-	2(100)	-	-	16(100)	1(14.3)	-	6(85.7)
Amikacin/ basil oil	-	1(50)	1(50)	3(18.75)	8(50)	5(31.25)	3(42.8)	-	4(57.14)
Ciprofloxacin/ basil oil	1(50)	-	1(50)	11(68.75)	-	5(31.25)	5(71.42)	-	2(28.6)

Total number of *klebsiella pneumonia* (2), *citrobacter freundii* (16), *pseudomonas aeruginosa* (7).

Key: S= Sensitive; R= Resistant; I =Intermediate

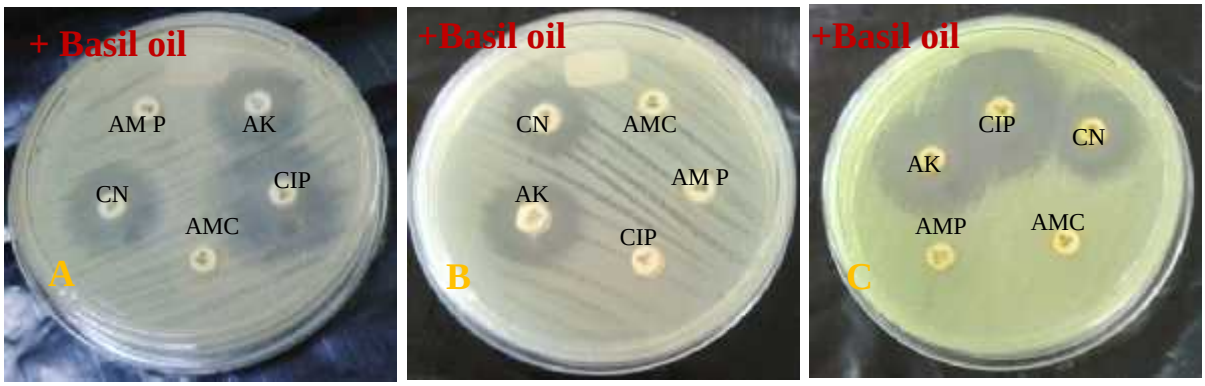

Fig (5): Photograph of effect of combination between basil oil and antibiotics against Gram negative bacterial isolates (Disc diffusion method)

(A) Photograph of effect of combination between basil oil and antibiotics against *klebsiella pneumonia*, (B) Photograph of effect of combination between basil oil and antibiotics against *citrobacter freundii*, (c) Photograph of effect of combination between basil oil and antibiotics against *pseudomonas aeruginosa*

Table (11) Effect of combination between basil oil and antibiotics against Gram positive bacterial isolates

Pair combinations	*Staph aureus*			*Co agulase negative staph*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Cefoxitin/ basil oil	1(11.11)	1(11.11)	7(77.8)	-	3(60)	2(40)
Clindamycin/ basil oil	5(55.55)	-	4(44.44)	4(80)	-	1(20)
Azithromycin/ basil oil	3(33.33)	1(11.11)	5(55.55)	-	3(60)	2(40)
Vancomycin/ basil oil	4(44.44)	3(33.33)	2(22.22)	-	-	5(100)
Gentamycin/ basil oil	2(22.22)	1(11.11)	6(66.7)	3(60)	1(20)	1(20)

Total number of staph aureus (9), coagulase negative staph (5)

Key: S = sensitive; R= Resistant; I intermediate

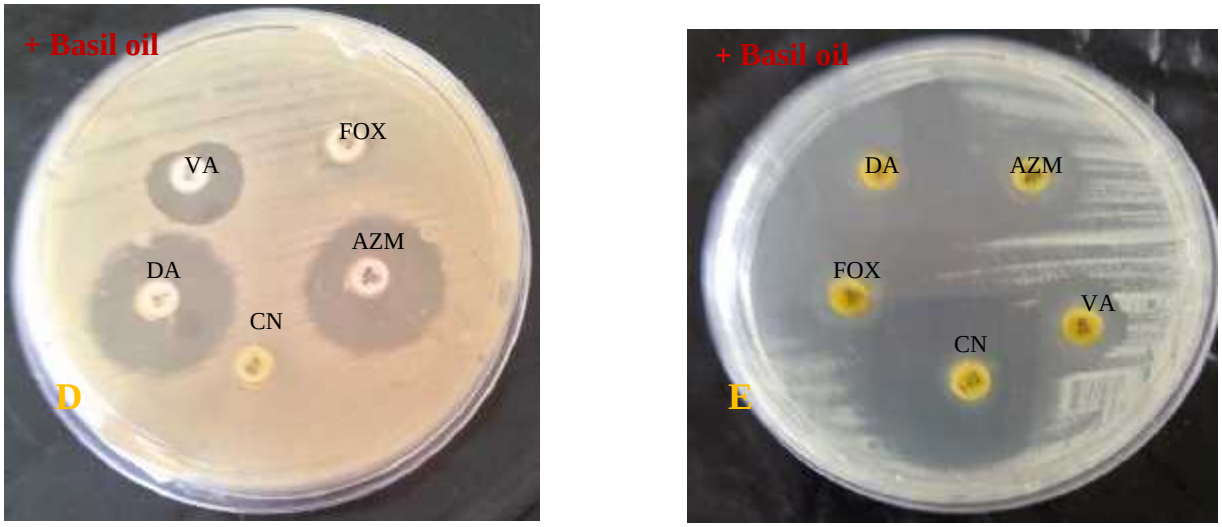

Fig (6): Photograph of effect of combination between basil oil and antibiotics on Gram positive bacterial isolates (Disc diffusion method)

(D) Photograph of effect of combination between basil oil and antibiotics against *staph aureus*, (E) Photograph of effect of combination between basil oil and antibiotics against *coagulase negative staph*

Table (12): Effect of combination between black seed oil and antibiotics against Gram negative bacterial isolates by disk diffusion method

Pair combinations	*Klebsiella pneumonia*			*Citrobacter freundii*			*Pseudomonas aeruginosa*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin/ black seed oil	-	-	2(100)	1(6.25)	-	15(93.75)	-	-	7(100)
Gentamycin/ black seed oil	-	1(50)	1(50)	-	5(31.25)	11(68.75)	3(42.8)	2(28.6)	2(28.6)
Amoxicillin-clavulanate/ black seed oil	-	-	2(100)	-	5(31.25)	11(68.75)	-	-	7(100)
Amikacin/ black seed oil	-	1(50)	1(50)	1(6.25)	3(18.75)	12(75)	5(71.42)	1(14.3)	1(14.3)
Ciprofloxacin/ black seed oil	1(50)	-	1(50)	11(68.75)	-	5(31.25)	6(85.7)	-	1(14.3)

Total number of *Klebsiella pneumonia* (2), *citrobacter freundii* (16), *pseudomonas aeruginosa* (7)

Key: S = sensitive; R= Resistant; I=intermediate

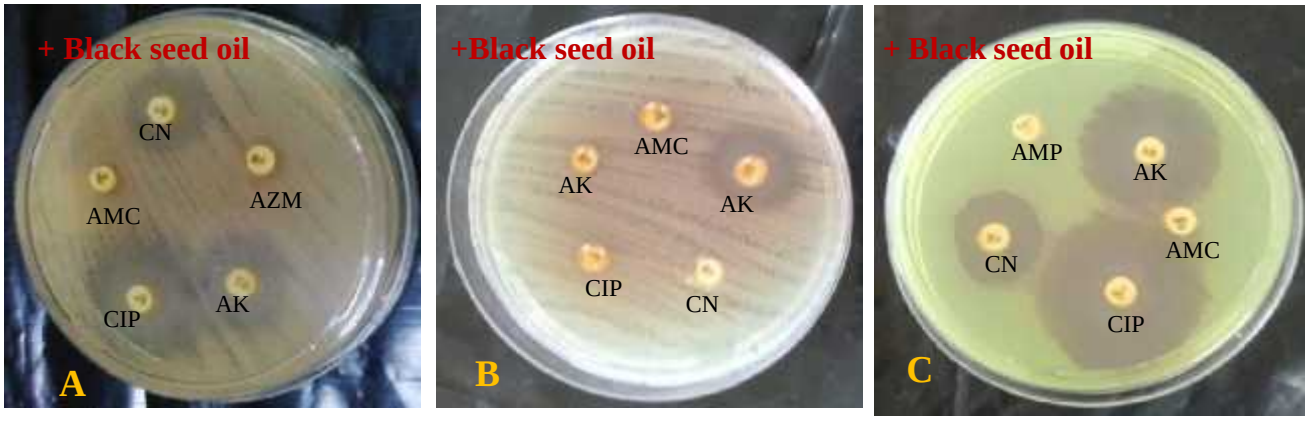

Fig (7): **Photograph of effect of combination between black seed oil and antibiotics against Gram Negative bacterial isolates (Disc diffusion method)**

(A) Photograph of effect of combination between Black seed oil and antibiotics against *klebsiella pneumonia*,(B) Photograph of effect of combination between Black seed oil and antibiotics against *citrobacter freundii*,(c) Photograph of effect of combination between black seed oil and antibiotics against *pseudomonasaeruginosa*

Table (13) Effect of combination between black seed oil and antibiotics against Gram positive bacterial isolates

Pair combinations	*Staph aureus*			*Co agulase negative staph*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Cefoxitin/ black seed oil	7(77.8)	-	2(22.22)	4(80)	-	1(20)
Clindamycin/ black seed oil	7(77.8)	-	2(22.22)	4(80)	-	1(20)
Azithromycin/ black seed oil	7(77.8)	-	2(22.22)	4(80)	-	1(20)
Vancomycin/ black seed oil	7(77.8)	-	2(22.22)	4(80)	-	1(20)
Gentamycin/ black seed oil	7(77.8)	-	2(22.22)	4(80)	-	1(20)

Total number of *staph aureus* (9), *coagulase negative staph* (5)

Key: S = sensitive; R= Resistant; I= intermediate

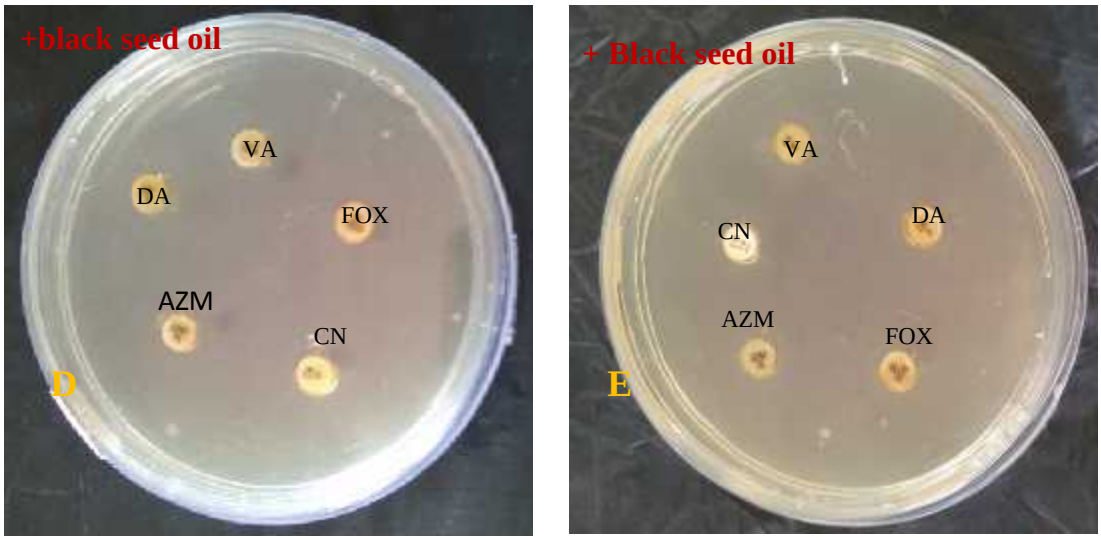

Fig (8): Photograph of effect of combination between black seed oil and antibiotics against Gram positive bacterial isolates (Disc diffusion method)

(D) Photograph of effect of combination between Black seed oil and antibiotics against *staph aureus*, (E) Photograph of effect of combination between Black seed oil and antibiotics against coagulase *negative staph*

Table (14): Effect of combination between rose marry oil and antibiotics against Gram negative bacterial isolates

Pair combinations	*Klebsiella pneumonia*			*Citrobacter freundii*			*Pseudomonas aeruginosa*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin/ rose marry	-	-	2(100)	-	-	16(100)	-	-	7(100)
Gentamycin /rose marry	-	1(50)	1(50)	1(6.25)	5(31.25)	10(62.5)	4(57.14)	1(14.3)	2(28.6)
Amoxicillin-clavulanate /rose marry	-	-	2(100)	6(37.5)	5(31.25)	5(31.25)	1(14.3)	-	6(85.7)
Amikacin/ rose marry	-	1(50)	1(50)	4(25)	6(37.5)	6(37.5)	4(57.14)	1(14.3)	2(28.6)
Ciprofloxacin/ rose marry	1(50)	-	1(50)	11(68.75)	-	5(31.25)	5(71.42)	1(14.3)	1(14.3)

Total number of *Klebsiella pneumonia* (2), *Citrobacter freundii* (16), *Pseudomonas aeruginosa* (7)

Key: S = sensitive; R= Resistant; I= intermediate

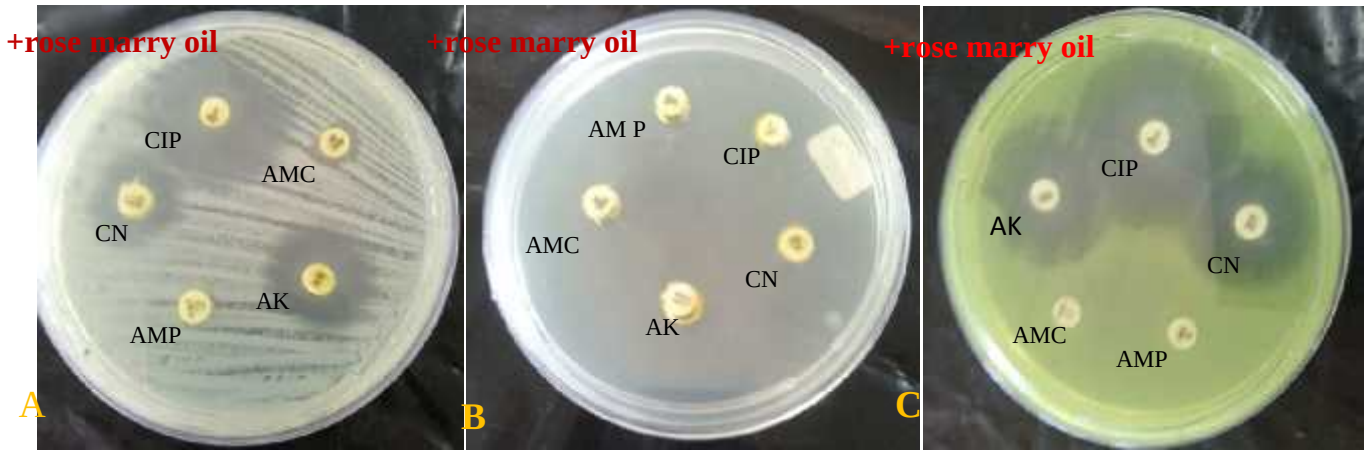

Fig (9): Photograph of effect of combination between rose marry oil and antibiotics against Gram negative bacterial isolates (Disc diffusion method)

(A) Photograph of effect of combination between Rose marry oil and antibiotics against *klebsiella pneumonia*, (B) Photograph of effect of combination between Rose marry oil and antibiotics against citrobacter *freundii*,(C) Photograph of effect of combination between Rose marry oil and antibiotics against *pseudomonas aeruginosa*

Table (15): Effect of combination between rose marry oil and antibiotics against Gram positive bacterial isolates

Pair combinations	*Staph aureus*			*Co agulase negative staph*		
	S (%)	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**
Cefoxitin/rose marry oil	3(33.33)	-	6(66.7)	4(80)	-	1(20)
Clindamycin/rose marry oil	3(33.33)	-	6(66.7)	4(80)	-	1(20)
Azithromycin/rose marry oil	4(44.44)	1(11.11)	4(44.44)	3(60)	1(20)	1(20)
Vancomycin/ rose marry oil	2(22.22)	7(77.8)	-	4(80)	-	1(20)
Gentamycin/ rose marry oil	2(22.22)	1(11.11)	6(66.7)	4(80)	-	1(20)

Total number of *staph aureus (9*), coagulase negative staph (5)

Key: S = sensitive; **R**= Resistant; **I** intermediate

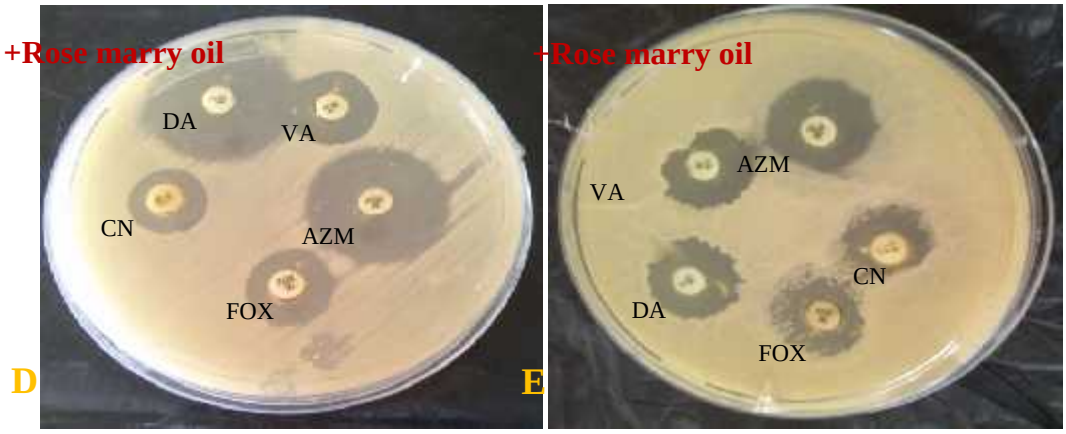

Fig (5.10): photograph of effect of combination between rose marry oil and antibiotics against Gram positive bacterial isolates (Disc diffusion method)

(D) Photograph of effect of combination between Rose marry oil and antibiotics against *staph aureus* (E) Photograph of effect of combination between Rose marry oil and antibiotics on *coagulase negative staph*

Table (16) Effect of combination between clove oil and antibiotics against Gram negative bacterial isolates

Pair combinations	*Klebsiella pneumonia*			*Citrobacter freundii*			*Pseudomonas aeruginosa*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin/ clove oil	-	-	2(100)	-	-	16(100)	1(14.3)	-	6(85.7)
Gentamycin/ clove oil	-	1(50)	1(50)	-	5(31.25)	11(68.75)	4(57.14)	1(14.3)	2(28.6)
Amoxicillin-clavulanate/ clove oil	-	-	2(100)	-	4(25)	12(75)	-	-	7(100)
Amikacin/ clove oil	-	-	2(100)	11(68.75)	1(6.25)	4(25)	5(71.42)	1(14.3)	1(14.3)
Ciprofloxacin/ clove oil	1(50)	-	1(50)	11(68.75)	-	5(31.25)	5(71.42)	-	2(28.6)

Total number of *Klebsiella pneumonia* (2), *Citrobacter freundii* (16), *Pseudomonas aeruginosa* (7*)*

Key: S = sensitive; R= Resistant; I = intermediate

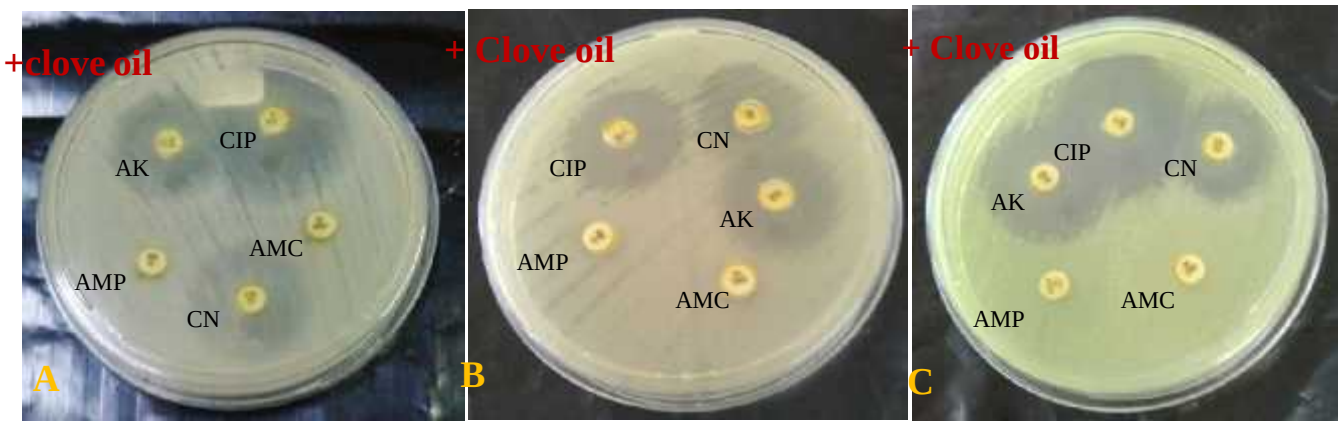

Fig (5.11): Photograph of effect of combination between clove oil and antibiotics against Gram Negative bacterial isolates (Disc diffusion method)

(A) Photograph of effect of combination between clove oil and antibiotics against *klebsiella pneumonia*, (B) Photograph of effect of combination clove oil and antibiotics against *citrobacter freundii*, (C) Photograph of effect of combination between clove oil and antibiotics against *pseudomonas aeruginosa*

Table (17) Effect of combination between clove oil and antibiotics against Gram positive bacterial isolates

Pair combination	*Staph aureus*			*Co agulase negative staph*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Cefoxitin/ clove oil	2(22.22)	-	7(77.8)	3(60)	-	2(40)
Clindamycin/ clove oil	3(33.33)	-	6(66.7)	4(80)	-	1(20)
Azithromycin/ clove oil	8(88.9)	-	1(11.11)	3(60)	-	2(40)
Vancomycin/ clove oil	1(11.11)	7(77.8)	1(11.11)	3(60)	1(20)	1(20)
Gentamycin/ clove oil	2(22.22)	1(11.11)	6(66.7)	3(60)	1(20)	1(20)

Total number of *staph aureus* (9), *coagulase negative staph* (5)

Key: S = sensitive; R= Resistant; I = intermediate

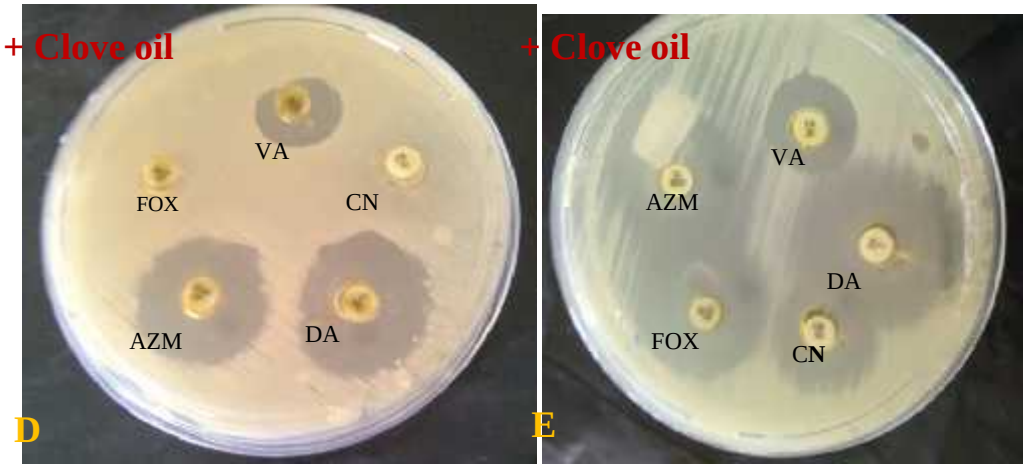

Fig (12): Photograph of effect of combination between clove oil and antibiotics against Gram positive bacterial isolates (Disc diffusion method)

(D) Photograph of effect of combination between clove oil and antibiotics on *staph aureus*, (E) Photograph of effect of combination between clove oil and antibiotics on *coagulase negative staph*

Table (18): Effect of combination between thyme oil and antibiotics against Gram negative bacterial isolates

Pair combinations	*Klebsiella pneumonia*			*Citrobacter freundii*			*Pseudomonas aeruginosa*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin/ thyme oil	-	-	2(100)	-	-	16(100)	-	-	7(100)
Gentamycin/ thyme oil	1(50)	-	1(50)	1(6.25)	11(68.7)	4(25)	5(71.42)	-	2(28.6)
Amoxicillin-clavulanate/ thyme oil	-	-	2(100)	-	4(25)	12(75)	-	-	7(100)
Amikacin/ thyme oil	-	1(50)	1(50)	7(43.75)	7(43.7)	2(12.5)	5(71.42)	2(28.6)	-
Ciprofloxacin/ thyme oil	1(50)	-	1(50)	11(68.75)	-	5(31.25)	7(100)	-	-

Total number of *Klebsiella pneumonia* (2), *Citrobacter freundii* (16), *Pseudomonas aeruginosa* (7)

Key: S = sensitive; R= Resistant; I= intermediate

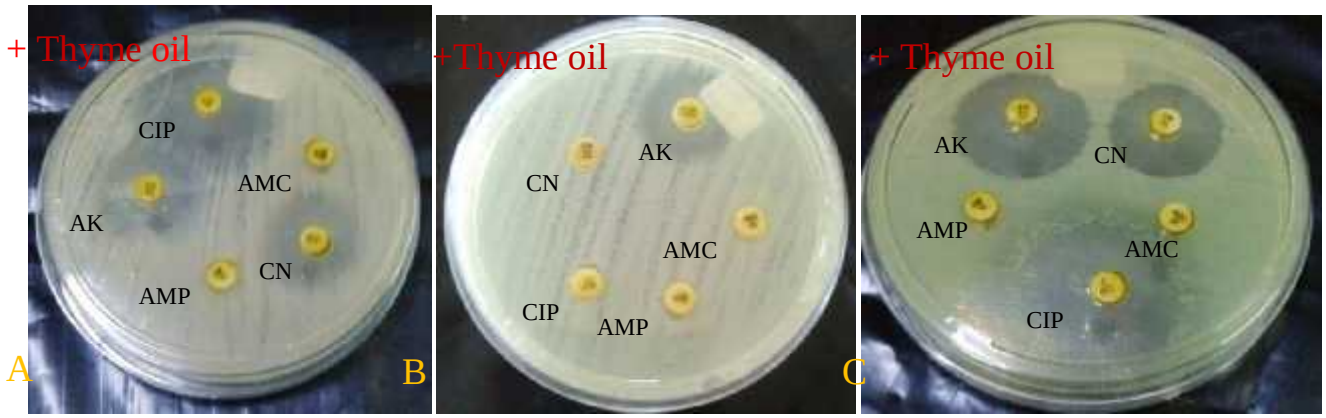

Fig (5.13): photograph of effect of combination between thyme oil and antibiotics against Gram Negative bacterial isolates (Disc diffusion method)

(A) Photograph of effect of combination between thyme oil and antibiotics against *klebsiella pneumonia* (B) Photograph of effect of combination between thyme oil and antibiotics against *citrobacter freundii* (C) Photograph of effect of combination between thyme oil and antibiotics against *pseudomonas aeruginosa*

Table (19) Effect of combination between thyme oil and antibiotics against Gram positive bacterial isolates

Pair combinations	*Staph aureus*			*Co agulase negative staph*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Cefoxitin/ thyme oil	7(77.8)	1(11.11)	1(11.11)	3(60)	-	2(40)
Clindamycin/ thyme oil	3(33.33)	-	6(66.7)	4(80)	-	1(20)
Azithromycin/thyme oil	7(77.8)	1(11.11)	1(11.11)	-	3(60)	2(40)
Vancomycin/ thyme oil	1(11.11)	6(66.7)	2(22.22)	-	-	5(100)
Gentamycin/ thyme oil	7(77.8)	-	2(22.22)	4(80)	-	1(20)

Total number of staph aureus (9)

Total number of co agulase negative staph (5)

Key: S = sensitive; R= Resistant; I intermediate

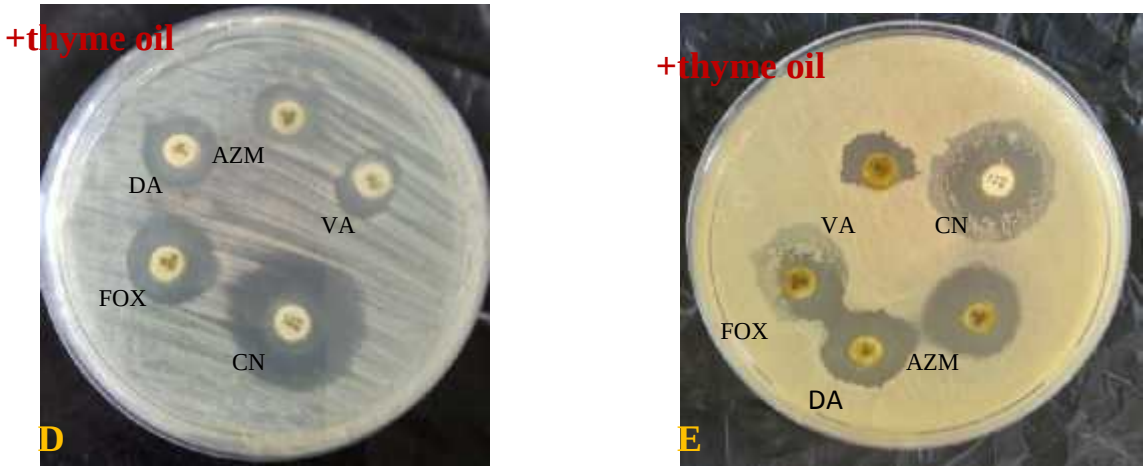

Fig (14) Photograph of effect of combination between thyme oil and antibiotics against Gram positive bacterial isolates (Disc diffusion method)

(D) Photograph of effect of combination between thyme oil and antibiotics against *staph aureus*, (E) photograph of effect of combination between thyme oil and antibiotics against *co agulase negative staph*

- Detection of beta lactamase mediated resistance

Beta lactamase production were detected using iodometric method The results show that all beta lactamase producing strains of *Klebsiella pneumonia* and *co agulase negative staph* (100%), (87.5%) of *Citrobacter freundii* (n=16), (80%) of *pseudomonas aeruginosa* (n=5) , (85.7%) of *staph aureus* (n=7) gave positive result by the iodometric method (table20and fig. 15).

Our results are agreement with Oberhofer et al. 1982 **[28]** who reported that all 169 (81. 6%) resistant *S. aureus* (n=207) isolates positive for β-lactamase test by iodometric method. He observed similar findings in *staph epidermidis, Haemophilus spp*. and *Neisseria spp*. and *Bacillus catarrhalis* isolates.

Table (20): Detection of β lactamase production by iodometric method

Bacterial isolates	Total no. of isolated bacteria	No of βlactam resistant isolates (%)	B lactamase test result	
			Positive no. (%)	Negative no. (%)
Citrobacter freundii	16	16(100%)	14(87.5%)	2(12.5%)
Klebsiella pneumonia	2	2(100%)	2(100%)	-
Pseudomonas aeruginosa	7	5(71.4%)	4(80%)	1(20%)
Staph aureus	9	7(77.7%)	6(85.7%)	1(14.3%)
Coagulase negative staph	5	4(80%)	4(100%)	-
Total	**39**	**34(100)**	**30(88.23%)**	**4(11.8%)**

Fig (15) Photograph of detection of beta lactamase activity by iodometric method

(A)Positive result of iodometric method, (B) negative Result of iodometric method

4- CONCLUSION

From this study it can be concluded that:

The most bacteria responsible for wound infections were *citrobacter freundii*, *staph aureus* and *pseudomonas aeruginosa*.

The most effective antibiotics were ciprofloxacin, Amikacin, Gentamycin against all gram negative bacterial isolates, the most effective antibiotics were Vancomycin on staph *aureus* and the most effective antibiotics were Clindamycin on *coagulase negative staph.*

Beta lactam antibiotics (Ampicillin, amoxicillin clavulanate, cefoxitin) were the most resistant antibiotics.

Essential oils possess antibacterial activity against gram positive bacterial isolates than gram negative bacterial isolates.

Combination between antibiotics and essential oils were more effective against different bacterial isolates than using both individually.

Iodometric method was accurate and gave satisfactory results in detection of beta lactamase enzyme in resistant isolates to beta lactam antibiotics.

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