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The antibacterial and synergistic potential of some Palestinian plant extracts against multidrug resistant *Staphylococcus aureus*

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Abstract
The present study was designed to screen *in vitro* antibacterial and synergistic activity of *Allium sativum, Ecballium elaterium, Pelargonium graveolens, Rosmarinus officinalis, Phagnalon rupestre & Ruta graveolens* plants against multidrug resistant *Staphylococcus aureus*. The active compounds were extracted from the dried aerial parts of plants with aqueous, 80% ethanol and methanol solvents by using Soxhlet extractor, and essential oils (EOs) which extracted from the fresh aerial parts of plant by using steam distillation. All extracts were screened for their antibacterial activity and synergistic effect in combination with known antimicrobial agents by using the disk diffusion method. The results revealed that, the average diameter of inhibition zones that resulted from the effect of plant extracts against the tested bacteria ranged from 8.33 to 12.66 mm, 10.66 to 13.33mm, 8.33 to 14.66 mm and 8.33 to 12.66 mm for aqueous, ethanol, methanol & EOs extracts, respectively. The extracts that showed antibacterial activity were subjected to minimum inhibitory concentration and minimum bactericidal concentrations assay; a micro-broth dilution assay was performed on 96-well plates using 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) as an indicator for bacterial growth. The average minimum inhibitory concentrations (MICs) values ranged from 12.5 to 25 mg/ml, 1.562 to 25 mg/ml, 1.562 to 25 mg/ml & 25to 50 µl/ml for aqueous, ethanol, methanol & EOs extracts, respectively. While minimum bactericidal concentrations (MBCs) values ranged from 50 to > 200 mg/ml, 100 to > 200 mg/ml, 25 to 200 mg/ml & 100 to > 200 µl/ml for aqueous, ethanol, methanol & EOs extracts, respectively. Synergistic activity of the plant extracts EOs when combined with antibiotics had different degree of synergism against *S. aureus*.
The results indicated that the possibility of concurrent use of these antimicrobial drugs and plant extracts in combination in treating infectious diseases caused by MDR S. aureus.

Keywords: Antibacterial, synergism, multidrug resistant, *Staphylococcus aureus*, medicinal plants

Introduction
Medicinal plants have been used as sources of medicine in virtually all cultures. During the last decade, the use of traditional medicine (TM) has expanded globally and is gaining popularity. People use herbal remedies due to their efficacy, tradition and their low cost. However, they often do not inform their physicians about their use of medicinal plants (Alonso-Castro *et al*., 2012) [11]. Medicinal plants are important elements of indigenous medical systems in Palestine as well as in other developing countries. Complementary and alternative medicine utilization in Palestine are common elsewhere, whereas other types were unique to this area (Abou Elkhair *et al*., 2010; Jouda *et al*., 2015a; Jouda *et al*., 2015b; Kichaoi *et al*., 2016; Elbashiti *et al*., 2016; Elbashiti, 2016) [1, 44, 45, 31, 29, 31].
There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases, which have led to the emergence of new bacterial strains that are multi-resistant which have resulted in increase in morbidity and mortality and creates enormous health problems. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics (Al-Sokari & El Sheikha., 2015; Elkhair., 2014; Davies & Davies., 2010) [9, 32, 25].
One strategy employed to overcome these resistance is the use of combination of drugs. The secondary metabolites from plant are good sources for combination therapy. There are a wide range of phytochemicals which act as multidrug resistance modifiers depicted (Hemaiswarya *et al*., 2008) [37].

In the present study, we have selected 6 medicinal plants (A*: Allium sativum (A. sativum),* B*: Ecballium elaterium (E. elaterium),* C*: Pelargonium graveolens (P. graveolens),* D*: Rosmarinus officinalis (R. officinalis),* E*: Phagnalon rupestre (P. rupestre)* & F*: Ruta graveolens (R. graveolens)*) to assess their antibacterial and synergistic effect with antibiotic drugs against multi-drug resistant *S. aureus***.**

Materials and Methods

Chemicals and Culture Media

Four types of media were used for carrying out this study Brain Heart Infusion Agar, Nutrient broth, Mueller Hinton Broth and Mueller-Hinton agar. Also ethanol and methanol used for extraction process. ampicillin, cefuroxime, cefotaxime, gentamicin, erythromycin, clindamycin, ofloxacin, nalidixic acid, norfloxacin, ciprofloxacin, amoxicillin-clavulanic acid, Ceflexin, Rifampicin & amikacin used as reference antibiotics.

Plant materials

The sex medicinal plants investigated in this study are collected from Gaza strip, khanyunes city- Absan alkaberah area in October 2014.

Microorganisms

The multi-drug resistant clinical isolate *Staphylococcus aureus* were obtained from microbiology department at Al-Shifa hospital, and were maintained on Brain Heart Infusion agar medium slant at 4 ºC until testing.

Preparation of Plant Extract

Preparation of Water Extract

The dried materials (20 g) were subjected to 150 ml distilled water for 8 h in a Soxhlet extractor. The extracts were concentrated under reduced pressure (rotary evaporator) then preserved at 4ºC until use (Boukhris *et al.,* 2013) [20].

Preparation of Methanolic Extract

Thirty gram of finely ground plant part powder was placed in porous bag made of muslin cloth, which was loaded into the main chamber of the Soxhlet. The extraction was carried out with methanol as extraction solvent in 1:10 ratio of powder to solvent at temperature 65 °C for 8 hours, and then the extract was filtered and allowed to evaporate in open air Albayrak, *et al.,* 2010) [6].

Preparation of Ethanolic Extract

Twenty gram of each plant parts were extracted separately with 150 ml of ethanol solvent for 8 hours using Soxhlet. The solvent was evaporated in oven at 37ºC for three days (Jameela *et al.,* 2011) [41].

Extraction of the Essential oils

Essential oil was extracted from 500 g of fresh and cleaned aerial parts of *A. sativum* (bulbs), *E. elaterium* (fruit), *P. graveolens* (shoots), *R. officinalis* (shoots), *P. rupestre* (shoots) & *R. graveolens* (leaf), by steam distillation method (Pokhrel *et al*., 2012; Hsouna & Hamdi, 2012; Abu-Al-Basal, 2010 & Tsao & Yin, 2001) [54, 38].

Preparation of Stock and Test solutions

One gram of each aqueous, ethanolic & methanolic extracts & 1ml of essential oils was carefully taken, and then each extract was made up to 5 mL by adding Dimethylsulfoxide (DMSO) and stored at 4°C until use. This formed the stock solution of 200 mg/ml (Mabrouk, 2012) [48].

Standardization of Inoculum

The optical density of each active culture was adjusted to 0.1 at 625 nm using fresh broth to give a standard inoculum of 10^6 colony forming units (cfu) per ml (Alzoreky & Nakahara, 2003) [10].

The Antibiotic Sensitivity Assay

The antibiotic sensitivity against *S. aureus* was determined using the disc diffusion method. Organisms were classified as sensitive, intermediate or resistant, based on the CLSI standards. Sixteen antibiotics were used in this study including: Ciprofloxacin (CIP), Ampicillin (AM), Cefotaxime (CTX), Nalidixic acid (NA), Norofloxacin (NOR), Cefuroxime (CXM), Cefaclor (CF or CEC), Ofloxacin (OFX), Cefalexin (CL or CN), Tetracycline (TE), Rifampicin (RIF), Amoxyclav (AMC), Gentamycin (GMN), Penicillin (P) & Oxacillin (OX).

Antimicrobial Activity Assays

Disc Diffusion Method

Standardized inoculums of *S. aureus* were introduced onto the surface of sterile Muller-Hinton agar (MHA) plates and a sterile cotton swab was used for even distribution of inoculums. After a few minutes, filter paper discs of 6 mm diameter were placed on the surface of inoculated and impregnated with 50 µL of known concentration of extracts (200 mg /ml) for aquatic, ethanolic, methanolic extracts & essential oils). Sterile paper discs containing Dimethyl sulfoxide alone was served as control. The plates were placed at 4ºC for 2 h and then subsequently incubated at 37º C for 24 h**.** After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm. For each test solution, three replicates were maintained (Gupta., *et al*, 2014; Casella *et al.,* 2013) [35, 12].

Determination of MIC and MBC by Microdilution Method

The plant extracts were dissolved in DMSO. Two-fold dilution series was prepared to achieve a decreasing concentration ranging from 200 to 0.390 mg/ml of each extract which was prepared in a 96-well microtiter plate. Overnight broth cultures of *S. aureus* were prepared, the final concentration in each positive well was adjusted to 10^6 CFU/ml and the plates were incubated at 37 ºC for 24 h. The MIC was defined as the lowest concentration of the plant extract at which the bacteria does not show visible growth. To determine MBC, broth was taken from each well and inoculated in MHA at 37 ºC for 24 h. The MBC is defined as the lowest concentration of the plant extract at which inoculated bacteria was totally killed. Amikacin and 10% DMSO solution served as positive and negative controls, respectively (Cheraif *et al,* 2007) [24].

Evaluation of the synergistic effect

The antibiotic filter paper disk of 6 mm in diameter were placed on the surface of inoculated and labeled MHA plates and impregnated with 50 µL of known concentration of extracts (200 mg /ml for aquatic, ethanolic, methanolic extracts & 200 µl/ml for essential oils). The plates were incubated at 37º C for 24 h. The diameters of cleared zones were measured and compared with that of the antibiotic alone (Elbashiti *et al*, 2011) [27].

All the experiments were done in triplicate. Normal saline was run as negative control along with sample analysis.

Statistical Analysis

All data were expressed as the mean ± standard deviation (SD) by measuring three independent replicates. One-way analysis of variance (SAS, 1990; ANOVA procedure) followed by Duncan's test was performed to compare means and to test the significance of differences between means obtained among the treatments at $p < 0.05$ level of significance using SPSS 18 software.

Results

Evaluation of antibiotics activity

The results of antibacterial susceptibility testing represented in table 4.1 showed that *S. aureus* (Figure 1 a & b) was highly resistant to many antibiotics.

Table 1: Effect of antibiotic reference standard on pathogenic bacteria (inhibition zone expressed by mm)

Antibiotic / Bacteria	CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN	P	OX	AK
S. aureus	0	0	0	*	0	0	0	0	0	*	*	0	*	7	0	22

*: not tested, mm: millimeter.

Fig 1.a: antibiotic susceptibility testing against *S. aureus* **Fig 1.b:** antibiotic susceptibility testing against *S. aureus*

Evaluation of Antibacterial Activity of Plant Extracts:

The results of antibacterial activity of Aquatic extracts, Ethanol extracts, Methanol extracts & Essential oils of all the six plants when tested individually for their antibacterial activity against *S. aureus* at 37 ºC are shown in Table 2 and Figures 2-7.

Table 2: Antibacterial Effect of different plant Extracts against *S. aureus* in mm

		Group	Inhibition Zones	± Standard Deviation	F	Sig
Staphylococcus aureus	Aquatic	A	10.00	1.00	2.549	0.086
		B	10.00	1.00		
		C	10.66	1.15		
		D	12.66	2.08		
		E	8.33	0.57		
		F	9.66	2.52		
	Ethanolic	A	11.66	0.57	1.196	0.368
		B	11.33	1.53		
		C	13.00	1.73		
		D	13.33	2.31		
		E	10.66	0.57		
		F	11.66	2.08		
	Methanolic	A	12.66	0.57	2.941	0.058
		B	10.66	1.15		
		C	11.00	1.73		
		D	14.66	4.62		
		E	9.66	1.53		
		F	8.33	1.53		
	Essential oil	A	12.66	0.57	3.116	0.050
		B	9.00	1.73		
		C	12.33	2.52		
		D	11.33	1.53		
		E	10.00	2.00		
		F	8.33	1.53		

(A*: Allium sativum,* B*: Ecballium eleterium,* C*: Pelargonium graveolens,* D*: Rosmarinus officinalis,* E*: P. rupestre &* F*: Ruta graveolens*).

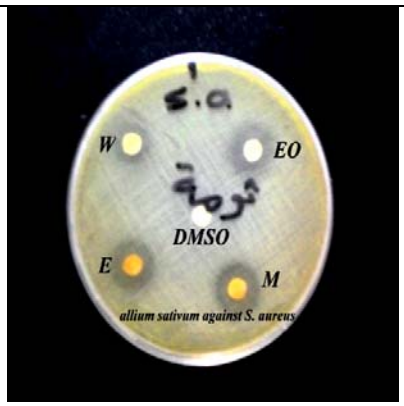

Fig 2: *A. sativum extracts against S. aureus*

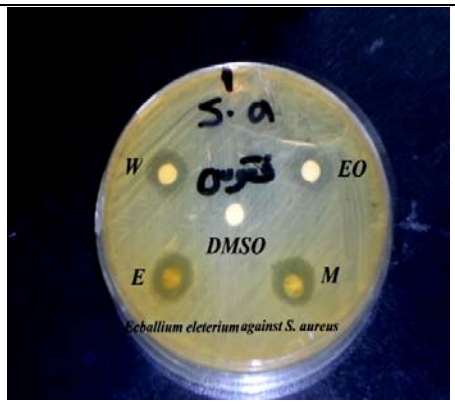

Fig 3: *E. elaterium extracts against S. aureus*

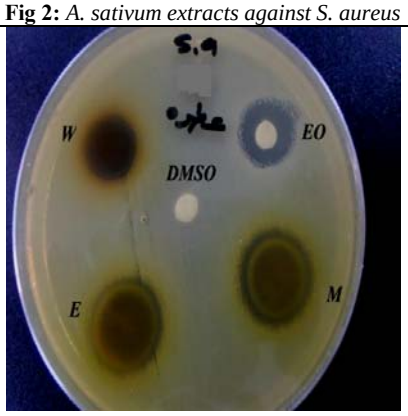

Fig 4: *Pelargonium graveolens extracts against S. aureus*

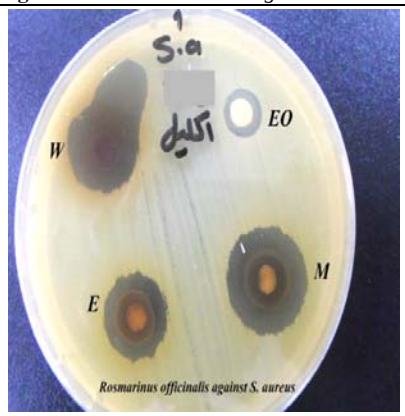

Fig 5: *R. officinalis extracts against S. aureus*

Fig 6: *P. rupestre extracts against S. aureus*

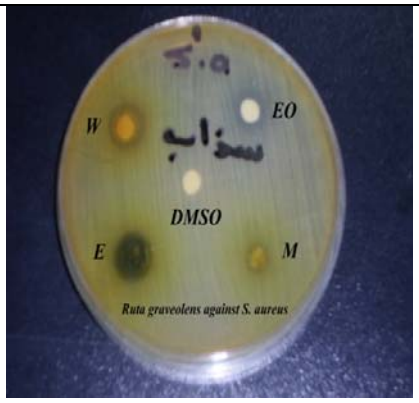

Fig 7: *Ruta graveolens extracts against S. aureus*

W: Aquatic, E: Ethanolic, M: Methanolic, EO: Essential oil

Synergistic Antibacterial Activity

The diameter of zone of inhibition of different combinations of plant extracts is represented in Tables (3-10) and Figures (8-15). Combinations of the extracts and antibiotics in several cases demonstrated synergistic, additive or antagonistic effects on microorganisms. Enlargement of the inhibition zones indicates a positive interaction (synergism) (Ahmad, & Aqil, 2007) [5].

Table 3: Synergistic activity of different plant extracts with different antibiotics against *S. aureus*

			CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
	Antibiotic alone		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**0**
	Extract alone												
Aquatic	A	10.00	13	0	0	0	0	0	0	0	0	10	0
	B	10.00	0	0	0	0	0	0	0	0	0	0	7
	C	10.66	10	8	9	8	9	9	10	8	8	12	0
	D	12.66	28	24	24	25	26	28	22	24	23	21	22
	E	8.33	0	0	0	0	0	0	0	0	0	8	0
	F	9.66	9	8	0	0	0	0	0	0	0	0	0

(A*: Allium sativum,* B*: Ecballium elaterium,* C*: Pelargonium graveolens,* D*: Rosmarinus officinalis,* E*: P. rupestre &* F*: Ruta graveolens).*

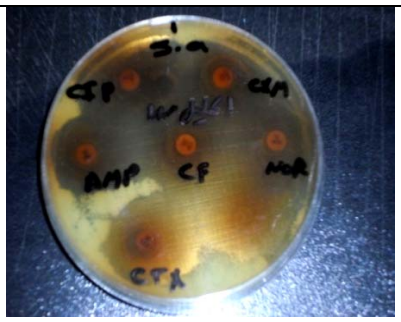

Fig 8: combination of *R. officinalis* aquatic extract with antibiotics against *S. aureus*

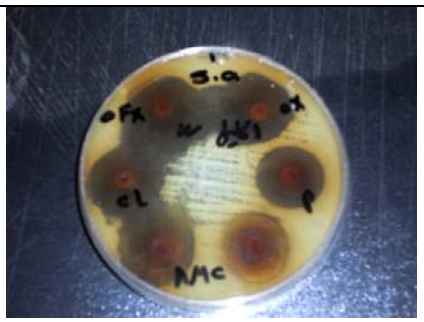

Fig 9: combination of *R. officinalis* aquatic extract with antibiotics against *S. aureus*

Table 4: The best synergism with aquatic extracts against *S. aureus*

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
	Group	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**
Aquatic	Means	28.00	24.00	24.00	25.00	26.00	28.00	22.00	24.00	23.00	21.00	22.00
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	F	240.60	524.80	855.90	913.50	995.10	1146.30	744.00	844.80	779.10	283.95	1131.36
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

D: *Rosmarinus officinalis*

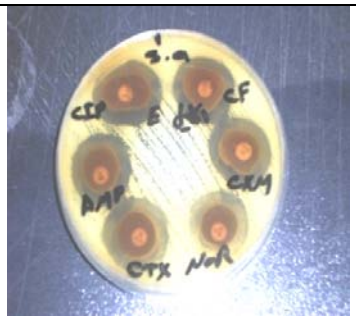

Fig 10: combination of *R. officinalis* ethanolic extract with antibiotics against *S. aureus*

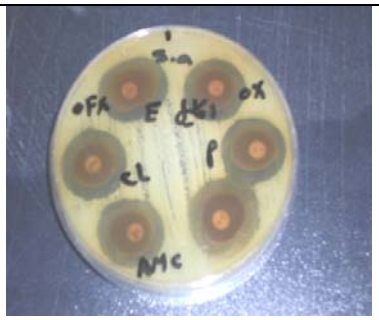

Fig 11: combination of *R. officinalis* ethanolic extract with antibiotics against *S. aureus*

Table 5: Synergistic activity of different plant extracts with different antibiotics against *S. aureus*

			CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
	Antibiotic alone		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**0**
	Extract												
Ethanolic	A	11.66	17	11	10	8	9	9	0	0	8	11	8
	B	11.33	12	0	0	0	8	8	0	0	9	10	0
	C	13.00	16	18	17	17	18	19	18	18	18	17	17
	D	13.33	22	23	23	22	22	23	21	22	21	22	22
	E	10.66	10	0	0	0	8	8	8	8	7	0	0
	F	11.66	16	19	16	17	12	12	13	16	14	15	14

(A*: Allium sativum,* B*: Ecballium elaterium,* C*: Pelargonium graveolens,* D*: Rosmarinus officinalis,* E*: P. rupestre &* F*: Ruta graveolens*).

Table 6: The best synergism with ethanolic extracts against *S. aureus*

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
	Group	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**
Ethanolic	Means	22.00	23.00	23.00	22.00	22.00	23.00	21.00	21.66	21.00	22.00	22.00
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.57	1.00	1.00	1.00
	F	52.50	445.35	403.20	399.00	103.70	121.70	358.20	472.90	114.40	202.68	371.55
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

D: *Rosmarinus officinalis*

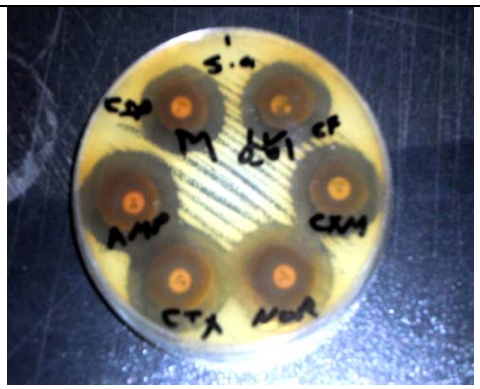

Fig 12: Combination of *R. officinalis* methanolic extract with antibiotics against *S. aureus*

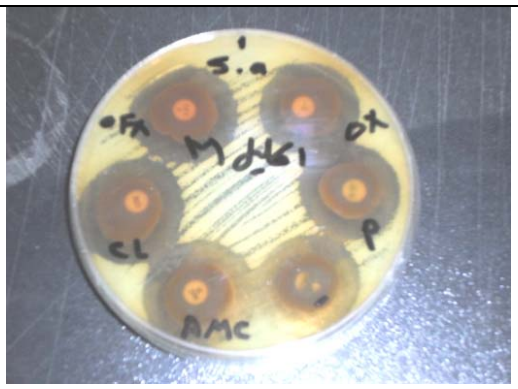

Fig 13: Combination of *R. officinalis* methanolic extract with antibiotics against *S. aureus*

Table 7: Synergistic activity of different plant extracts with different antibiotics against *S. aureus*

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Methanolic	Antibiotic alone	0	0	0	0	0	0	0	0	0	7	0
	Extract											
	A 12.66	9	8	9	9	10	9	8	9	9	11	8
	B 10.66	13	10	0	0	11	11	10	11	10	12	12
	C 11.00	17	15	14	15	16	14	15	15	17	14	15
	D 14.66	25	28	28	27	25	25	27	24	26	25	25
	E 9.66	12	8	9	8	8	9	0	0	8	9	8
	F 8.33	9	8	8.5	8	8	9	8	8	9	10	11

(A*: Allium sativum,* B*: Ecballium elaterium,* C*: P. graveolens,* D*: Rosmarinus officinalis,* E*: P. rupestre* & F*: Ruta graveolens).*

Table 8: the best synergism with methanolic extracts against *S. aureus*

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Methanolic	Group	D	D	D	D	D	D	D	D	D	D	D
	Means	25.00	28.00	28.00	27.00	25.00	25.00	27.00	24.00	26.00	25.00	25.00
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	F	110.90	187.70	311.19	298.68	129.60	118.10	296.16	229.56	150.50	104.10	121.70
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

D: *R. officinalis*

Evaluation of Antibacterial Activity of Plant Essential oils

The diameter of zone of inhibition of different combinations of plant essential oils are represented in Tables (9 & 10) and Figures (14 & 15).

Fig 14: Combination of: *P. graveolens* EO with antibiotics against *S. aureus*

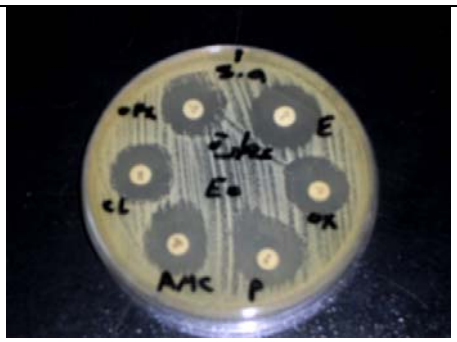

Fig 15: Combination of: *P. graveolens* EO with antibiotics against *S. aureus*

Table 9: Synergistic activity of different plant essential oils with different antibiotics against *S. aureus*

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Essential Oils	Antibiotic alone	0	0	0	0	0	0	0	0	0	7	0
	Extract											
	A 12.66	11	10.5	10	11	10	11	11	10	11.5	11	11
	B 9.00	0	0	0	0	0	0	0	0	0	0	0
	C 12.33	19	18	17.5	18.5	17	16	18	20	19.5	18	18
	D 11.33	14	12.5	13.5	12	13	15	15	14.5	15	14	14
	E 10.00	0	0	0	0	0	0	0	0	0	10	0
	F 8.33	0	0	0	7	0	0	8	8	10	7	7

(A*: Allium sativum,* B*: Ecballium elaterium,* C*: P. graveolens,* D*: Rosmarinus officinalis,* E*: P. rupestre* & F*: Ruta graveolens*).

Table 10: The best synergism with different plants essential oils against *S. aureus*

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Essential oil	**Group**	C	C	C	C	C	C	C	C	C	C	C
	Means	19.00	18.00	17.50	18.50	17.00	16.00	18.00	20.00	19.50	18.00	18.00
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	F	426.40	372.40	370.00	292.66	349.60	369.60	255.00	283.38	283.35	160.94	302.77
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

C: *P. graveolens*

Determination of MIC & MBC

Extracts were tested against the *S. aureus* isolate for their inhibitory activity, using a common broth microdilution method in 96 multiwell microtiter plates in two-fold dilution series of these extracts were prepared: 200, 100, 50, 25, 12. 5. 6.25, 3.125, 1.562, 0.781 & 0.390 (mg/ml for the aquatic, ethanolic & methanolic extracts and µl/ml for the essential oils), in triplicate and the average of the obtained minimum inhibitory concentrations (MICs) & minimum bactericidal concentrations (MBCs) is listed in Tables 11-16.

Table 11: The MICs & MBCs of *A. sativum* extracts against *S. aureus*

Scientific name of the plant used		***S. aureus***	
		MIC	MBC
Allium sativum	Aquatic mg/ml	50	200
	Ethanolic mg/ml	25	200
	Methanolic mg/ml	25	200
	Essential oil µl/ml	25	200

Table 12: The MICs & MBCs of *Ecballium eleterium* extracts against isolated bacteria

Scientific name of the plant used		***S. aureus***	
		MIC	MBC
Ecballium eleterium	Aquatic mg/ml	25	200
	Ethanolic mg/ml	25	200
	Methanolic mg/ml	25	200
	Essential oil µl/ml	50	> 200

Table 13: The MICs & MBCs of *P. graveolens* extracts against isolated bacteria

Scientific name of the plant used		***S. aureus***	
		MIC	MBC
P. graveolens	Aquatic mg/ml	12.5	> 200
	Ethanolic mg/ml	1.562	100
	Methanolic mg/ml	1.562	100
	Essential oil µl/ml	25	200

Table 14: The MICs & MBCs of *Rosmarinus officinalis* extracts against isolated bacteria

Scientific name of the plant used		***S. aureus***	
		MIC	MBC
Rosmarinus officinalis	Aquatic mg/ml	12.5	50
	Ethanolic mg/ml	6.25	> 200
	Methanolic mg/ml	1.562	25
	Essential oil µl/ml	50	200

Table 15: The MICs & MBCs of *Phagnalon rupestre* extracts against isolated bacteria

Scientific name of the plant used		***S. aureus***	
		MIC	MBC
Phagnalon rupestre	Aquatic mg/ml	12.5	200
	Ethanolic mg/ml	3.125	200
	Methanolic mg/ml	3.125	200
	Essential oil µl/ml	25	200

Table 16: The MICs & MBCs of *Ruta graveolens* extracts against isolated bacteria

Scientific name of the plant used		***S. aureus***	
		MIC	MBC
Ruta graveolens	Aquatic mg/ml	25	200
	Ethanolic mg/ml	3.125	100
	Methanolic mg/ml	3.125	100
	Essential oil µl/ml	50	> 200

Discussion

The usage of medicinal plants for primary health care needs by millions of people in developing world is still occupying a prominent position. The folk remedies are considered readily available, cheap and time tested (Pandikumar *et al*., 2011) [53].

In this study, different extracts of *A sativum, E. elaterium, P. graveolens, R. officinalis, P. rupestre & R. graveolens* showed significant antibacterial activity against multidrug resistant (MDR) *S. aureus* as assessed by the diameter of zone of inhibition of the extracts. Although, the low values recorded for some plant extracts may be attributed to the fact that the extracts being in crude form, contain very small amounts of bioactive compounds. At the same time, several workers have reported bioactivity of crude extracts of medicinal plants within such range of diameter zone of inhibition (Karmegam, *et al*., 2008) [46].

The aqueous extract of *A. sativum* possesses significant antibacterial activity against *S. aureus*. Similar results were obtained by Gupta *et al* (2015) [35], where they showed a strong antimicrobial activity of *A. sativum* aquatic extract against MDR clinical isolates *S. aureus* found in human urine in cases of urinary tract infection. The ethanolic extracts of *A. sativum* had a lower inhibitory activity against *S. aureus* than that of the water extract. This is in conformity with the work of Arekemase *et al* (2013) [12], in which the inhibitory effect of garlic extracts on the growth of *S. aureus, B. subtilis*, *E. coli, P. aeruginosa and K. pneumoniae* was reported. They discovered that ethanolic extract of garlic produced higher antimicrobial activity than the water extract of the plant against *E. coli*, *P. aeruginosa and K. pneumoniae*, while *S. aureus and Bacillus subtilis* showed least sensitivity. Meanwhile, the methanolic extract of garlic bulb was also effective against *S. aureus* under study. Similar results were obtained by Gaherwal *et al* (2014) [33], where they showed that The methanolic extract of garlic bulb was also effective against *E. coli, S. aureus and Salmonella enterica* serovar Typhi. which had maximum inhibition zone against *S. aureus* followed by *E. coli* and then *Salmonella* Typhi. Also, similar results were obtained by Gull *et al* (2012) [36], in which the inhibitory effect of aqueous, methanolic & ethanolic extracts of *A. sativum* had been assayed separately against drug resistant *S. aureus*. where they showed that the tested isolate was susceptible to garlic aqueous, methanolic and ethanolic extracts.

In general, the inhibitory activity of essential oils was greater than that of aquatic & ethanolic extracts. The EO of *A. sativum* bulbs was also effective against *S. aureus*. Our results are in agreement with the results obtained by Sharma *et al* (2013) [58], in which the antibacterial activity of *A. sativum* rhizome essential oil had been assayed against foodborne pathogens including *S. aureus* & *E. coli*. Also, our results are in agreement with the result obtained by Viswanathan *et al* (2014) [61], in which the antibacterial activity of *A. sativum* essential oil showed a potent antimicrobial activity against *S. aureus*.

The antimicrobial activity of *A. sativum* bulbs extracts suggested by Meriga *et al* (2012) [49], suggest that, these extracts contain effective phytochemicals responsible for the inhibition of microorganisms. The antibacterial activity of garlic is widely attributed to its phytochemical (Allicin), which is an important constituent of garlic interferes with RNA production and lipid synthesis.

The aquatic extract of *E. eleterium* shown a good inhibitory activity against *S. aureus*. In contrast with Dogruoz *et al* (2008) [26], which stated that the antibacterial activity of the aqueous extract of *E. elaterium* was not active against *S. aureus* at concentration of 0.001mg/ml. The reason for this variation could be assumed to low concentration of the aquatic extract of *E. eleterium* examined, difference in the extraction method & genetic differences between the *E. eleterium* and the clinical S. *aureus* isolate used in this study. Also, the ethanolic and methanolic extracts of *E. eleterium* fruits showed a good antibacterial activity against *S. aureus* in agreement with (Adwan *et al*., 2011 and Koca *et al*., 2010) [3, 47] respectively.

To the best of our knowledge, the antibacterial activity of *P. graveolens* extracts & EO growing in Gaza strip has never been reported. The aquatic extract of *P. graveolens* in general showed a high inhibitory activity against *S. aureus*. This work confirmed what is reported that aquatic extract of *P. graveolens* show antibacterial activity and disagree with the article reported that, the aquatic extracts remained inactive against *S. aureus* in the range of the used concentration 4 mg/wells (Hsouna & Hamdi., 2012) [38]. Meanwhile, the methanolic extract of *P. graveolens* exhibited a high antibacterial activity against *S. aureus* at the concentration of 10µl / disc. Our results were in agreement with the results of an earlier study (Boukhris *et al*., 2013) [20], in which the antibacterial activity of *P. graveolens* methanolic extract against *S. aureus* exhibit bactericidal effects. Essential oil of *P. graveolens* also showed a good antibacterial activity against *S. aureus*. Similar results were obtained by Boukhris *et al* (2013) [20], Ghannadi A *et al* (2012) [23], where they showed that, the *P. graveolens* EO were active against *S. aureus*.

Our results showed that, the antibacterial activity of the *R. officinalis* aquatic extract had high antibacterial activities against *S. aureus.* These results are consistent with previous reports on *R. officinalis* aquatic extracts regarding *S. aureus* (Jordan *et al* (2012) [43].

R. officinalis ethanolic extract showed antibacterial activity against *S. aureus.* According to Qabaha (2013) [55], *R. officinalis* ethanolic extract exhibit significant antimicrobial activity against *S. aureus* at concentration of 12.5 mg ml^{-1}. The methanolic extract exhibited varying degree of antibacterial activities against *S. aureus* in difference with (Irshaid *et al*.,2014) [39], who stated that *R. officinalis* methanolic extract exhibit high antibacterial effects against *P. aeruginosa*, *E. coli* & *S. aureus*. As the methanolic extract of *R. officinalis*, the essential oil exhibited moderate antibacterial activities against *S. aureus* in agreement with many other studies reported on this plant (Barbosa *et al*., 2015) [13].

This is the first time where the antibacterial effects of Palestinian *R. graveolens* aquatic extract against *S. aureus* were estimated.

Ruta graveolens ethanolic extract showed good antibacterial activity against *S. aureus*. Our findings are consistent with previous reports (Valsaraj *et al*., 1997) [60], which stated that *R. graveolens* ethanolic extract has antibacterial effect against *S. aureus. Ruta graveolens* methanolic extract has a moderate antibacterial activity against *S. aureus*. As is in the current study, previous study has reported the good antibacterial activity of methanolic extracts of *R. graveolens* on different microorganisms (Ivanova *et al*., 2005) [40]. The results revealed that R. *graveolens* EO also exhibited moderate antibacterial activities *S. aureus*. This results were in agreement with many other studies reported (Al-Shuneigat *et al*., 2015) [8] which investigated the Chemical Composition and the Antibacterial properties of *R. graveolens* L. EO Grown in Northern Jordan. In addition, in agreement with our

results Orlanda & Nascimento (2015) [52] whom reported that, the EO from *R. graveolens* had the highest antibacterial activity against *S. aureus*

The results revealed that *P. rupestre* aquatic and methanolic extracts had good antibacterial activities against *S. aureus.* These results are consistent with previous reports (Ali-Shtayeh *et al*., 1998) [7], in which *P. rupestre* aquatic and methanolic extract extracts showed good antibacterial activities against *S.* aureus*, E. coli, K. pneumoniae* and *P. aeruginosa*. The results of antibacterial assays revealed that this methanolic plant extract exhibited varying degree of antibacterial activities against *S. aureus.* Meanwhile, *P. rupestre* EO exhibited low antibacterial activities against *S. aureus*. Almost, there are no articles on the antibacterial activity of *P. rupestre* EO published in PubMed.

The inability of plant to diffuse through the Mueller-Hinton Agar Media illustrate the weakness of antibacterial effect of the plant extract using agar disk diffusion method. This impairment in drug diffusion is a major limitation in the evaluation of the antimicrobial effects of plant extracts using the agar diffusion method (Adwan & Mhanna., 2009) [4].

Synergistic effect resulting from the combination of antimicrobial agents with crude plant extracts was verified for most plants. The association of natural products such as plant extracts and antibiotics constitutes an alternative in the fight against MDR bacteria. Significant synergistic effects were noted with both *P. graveolens* and *R. officinalis* extracts when they were associated with several antibiotics. Such effects might be due to the action of the active compounds or possible inhibition of one or more mode of bacterial resistance mechanisms by other compounds of the extracts. *Pelargonium graveolens* and *R. officinalis* followed by *R. graveolens* ethanolic extracts alone or in combination are promising in the development of phytomedicine. It can be used alone or in combination with the antibiotics against MRSA. The synergistic effects that resulting from the combination of *R. officinalis* aquatic extract with CL & P against MRSA are compatible with results of study of Adwan & Mhanna., (2009) [4]. The lowest synergistic effects was observed with DNA synthesis inhibitors (CIP when combined with *E. elaterium* ethanolic extract and *P. rupestre* methanolic and *R. graveolens* aquatic & methanolic extracts and OFX when combined with *R. graveolens* ethanolic extract) and was observed with cell wall synthesis inhibitors (AM when combined with *R. graveolens* methanolic extract & AMC when combined with *R. graveolens* methanolic extract and EO and was observed also in case of P & OX combined with *R. graveolens* methanolic extract), this is obviously due to the fact their target are localized in the bacterial cell coat. However, the synergistic effects observed indicate that active compounds of the extracts could also present different mode(s) of action from those of the studied antibiotics. But other researchers study the effect of combination of plant extracts with antimicrobial agents against MRSA like El-Kalek & Mohamed., (2012) [30] which Screenings of Synergistic effect of certain medicinal plants and amoxicillin against some clinical isolates of MRSA. Results revealed that, all of tested plants possess a degree of antibacterial activity toward MRSA strains under study, but each of lemon grass oil, Cardamom oil and T*hymus vulgaris* extract possess highly degree of antibacterial activity towards (MRSA), lemon grass oil showed better inhibitory effects than others. The activity of Amoxicillin against MSSA (C.I), MSSA (ATCC) and 15 MRSA strains tested were from 15 to 28 mm (inhibition zone), three strains of MRSA (M2, M16 and M18) showed less susceptibility to amoxicillin. When Amoxicillin was combined with lemon grass oil, Cardamom oil and *Thymus vulgaris* extracts, the inhibition zones were increased. The results also revealed that, M2, M16 and M18 became sensitive to each combination, the results showed significantly increase in activity of Amoxicillin when combined with tested plants.

The broth microdilution method has provided a potentially useful technique for determining MICs & MBCs of large numbers of test samples. It is advantages over diffusion techniques include increased sensitivity for small quantities of extract which is important if the antibacterial is scarce as in the case for many natural products, some researchers however, have reported MICs & MBCs values obtained by the agar diffusion method, although high activity in the disk diffusion assay does not necessarily correlate to low MIC & MBC values in the microtiter plate method (Ncube *et al.,* 2008) [50]. This finding were agreed with our results in which the MIC of *R. graveolens* methanolic extract against *S. aureus* was 3.125 mg/ml while the inhibition zone diameter was 8.33 mm.

The results of MIC and MBC values showed that *A. sativum, E. elaterium, P. graveolens, R. officinalis, P. rupestre & R. graveolens* had potential inhibitory activity against *S. aureus*. This activity could be attributed to the active components present in plant extracts, which might be involved in some type of antibacterial synergism with the other active compounds. The MBC value of the *E. elaterium* EO*, P. graveolens* aquatic extract*, R. officinalis* ethanolic extract and *R. graveolens* EO against *S. aureus* used in the present study was not possible to determine against *S. aureus*, because the concentration of stock solution of extracts was beyond the concentration of MBC.

In general, these results showed that all of the studied plants are potentially a rich source of antimicrobial agents. However, the plants differ significantly in their activity against *S. aureus*. The main target of the hydrophobic compounds is the cell membrane. They lead to a cell membrane damage causing increased membrane permeability, ions leakage, and inhibition of different enzymes and proteins.

The bactericidal and fungicidal activities of the essential oil of *P. graveolens* could be explained by the presence of high concentrations of oxygenated relatively stable monoterpenic primary alcohols. β-Citronellol and geraniol, the two major monoterpenes component of Geranium oil, have reported antimicrobial activity (Boukhris *et al*., 2013 & Rosato *et al*., 2007) [21, 56].

The activity of the oils would be expected to relate to the composition of the plant essential oils and possible synergistic interaction between components (Ghannadi *et al*., 2012) [23]. he methanol and water extracts of flowers and leaves did show antimicrobial activity, with the flower extracts being overall more active than the leaf extracts. This activity is most likely associated with the phenolic compounds in these extracts that can effect cellular membranes altering their permeability and release of intracellular constituents (e.g. ribose and Na glutamate), but also interfere with membrane functions (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity) (Boukhris *et al.,* 2013) [21].

The relative bactericidal effectiveness of *R. officinalis* EO may come from the synergic effect of α-pinene and camphor highly represented in these oils (Zaouali *et al*., 2010) [62]. Also Jordán *et al*., (2013) [42] described The chemotypes which correspond eucalyptol-α-pinene-camphor, eucalyptol-

camphor-α-pinene, camphor-eucalyptol-α-pinene, camphor-α-pinene-eucalyptol and α-pinene-eucalyptol-camphor. All the chemotypes showed strong antibacterial activity against four food-borne pathogens (Gram negative: *S. typhimurium* and *E. coli* and two Gram positive: *L. monocytogenes* and *S. aureus*). The levels of antimicrobial activity in Tunisian *R. officinalis* oils were similar to those reported for *R. officinalis* from Iran, exhibiting a high antimicrobial activity against *E. coli, S. aureus and Listeria monocytogenes* due mainly to the dominance of borneol followed by camphor and verbenone respectively. The Turkish *R. officinalis* oil possesses a moderate antibacterial activity attributed to the high content of 1.8-cineole, the low content of camphor and verbenone, respectively. A weak activity was reported for samples from Sardinia dominated by α-pinene, camphene, verbenone, bornyl-acetate, camphor and borneol tested against *S. aureus, S. epidermidis, E. coli, P. aeruginosa* (Zaouali *et al*., 2010) [62].

Also Santoyo *et al*., (2005) [57] & Okoh *et al*., (2010) [51] attributed the antimicrobial property of the essential oil of *R. officinalis* to the presence of α-pinene, β-pinene, 1,8-cineole & borneol as the major components. These compounds possess strong antibacterial and antimicrobial activities, and camphor and verbinone being the most potent. These chemical components exert their antimicrobial activity on microorganisms through the disruption of bacteria membrane integrity. Another important characteristic of essential oils is their hydrophobicity which enables them to penetrate lipid components of bacterial cell membrane, disrupting the cell structure and rendering them more permeable resulting in leakages of critical molecules from within the cell and eventual death of the bacteria cells (Okoh *et al*., 2010) [51].

Conclusion & Recommendations

- The overall results of the present work provide baseline information for the possible use of the studied plant extracts in the treatment of *S. aureus* infections. In addition to these antibacterial activities, the data reported herein indicated that possible combinations of the extracts of *A. sativum, E. eleterium, P. graveolens, R. officinalis, P. rupestre & R. graveolens* plants with several antibiotics could be used in the control of *S. aureus*. Our results support the use of these plants in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search for new drugs.
- The tested crude extract from *A. sativum, E. eleterium, P. graveolens, R. officinalis, P. rupestre & R. graveolens* have proved to be promising treating agents against the tested *S. aureus*, but it needs to be concentrated and furthermore evaluated. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized.
- A wider study is needed to identify the effective components, the mode of action and the possible toxic effect *in-vivo* of these ingredients. The maximum benefit can be achieved when the pharmacokinetics of natural product and the antibiotic combination match. The optimal ratio and dosing regimens should be explored for higher efficacy and decreased toxicological profiles.

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