Functional Plant Science and Biotechnology ©2011 Global Science Books

The Antibacterial and Synergistic Effects of Some Palestinian Plant Extracts on *Escherichia coli* and *Staphylococcus aureus*

Tarek A. Elbashiti[1*] • Abdelraouf A. Elmanama[2] • Atef A. Masad[1]

[1] Department of Biotechnology, Islamic University of Gaza, Palestine
[2] Department of Medical Technology, Islamic University of Gaza, Palestine
Corresponding author: * **tbashiti@iugaza.edu.ps**

ABSTRACT

The antimicrobial activity and synergistic effect of some local plant extracts were evaluated against *Escherichia coli* and *Staphylococcus aureus*. Seven crude extracts from five plants obtained through four different extraction methods were screened and tested against *E. coli* and *S. aureus*. Extracts from *Cakile maritima* (roots and shoots), *Cakile maritima* (seeds), *Mesembryanthemum crystallinum* (whole plant), *Atriplex halimus* (leaves), *Withania somnifera* (leaves), *Marrubium vulgare* (stem and leaves) were tested. There was no antibacterial activity in any plant extracts against *E. coli* except for *C. maritima* (seeds) when extracted by ethanol with an inhibition zone = 13 mm. However, antibacterial potentials were observed against *S. aureus* when treated with extracts of *W. somnifera* (leaves) with an inhibition zone = 25 mm, *M. vulgare* (stems) with an inhibition zone = 15 mm and *M. vulgare* (leaves) with an inhibition zone = 13 mm, all of which were extracted by ethanol. The synergistic effect of plant extracts and antibiotics showed promising results against antibiotic-resistant bacteria. The results obtained with *E. coli* were particularly interesting since it was inhibited by antibiotics combined with *C. maritima* (roots, shoots and seeds), *M. crystallinum* (whole plant), *M. vulgare* (stem and leaves) extracts at least in one extraction method (ethanol for 8 h). This inhibition was not observed with the individual plant extracts alone but when they were used with the ineffective antibiotics. Some of the extracts showed a synergistic activity when tested against *S. aureus*. However, when *A. halimus* (leaves) were extracted by water reflux and *C. maritima* (seeds), *W. somnifera* (leaves) and *M. vulgare* (stem) were extracted by methanol for 5 days, they showed no synergistic effect. Overall, the highest synergistic effect was observed when the plant extracts were treated with tetracycline and minocycline against both *E. coli* and *S. aureus*.

Keywords: antimicrobial, medicinal plants, plant extract, synergism

INTRODUCTION

Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens (Deans and Ritchie 1987; Kumar *et al*. 2007). Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. An antibiotic resistant bacterium is a threat which is becoming increasingly common. Incidence figures in some hospitals have shown that more than 40% of *S. aureus* strains are now resistant to methicillin, abbreviated as MRSA (methicillin-resistant *Staphylococcus aureus*) (Lesse 1995). Therefore, the development of new antimicrobial agents for the treatment of bacterial infections is of increasing interest. Although pharmaceutical industries have produced a number of new antimicrobial drugs in the last few years, resistance to these drugs by microorganisms has increased rapidly. The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatment. In the last few years a number of studies have been conducted to verify the effectiveness of plant extracts against bacterial infections (Perumal 2005; Aboaba *et al*. 2006; Bassam *et al*. 2006; Prashanth *et al*. 2006; Owen and Palombo 2007). Many plants have been used because of their antimicrobial traits of compounds which are synthesized in the plant's secondary metabolism. These products are known by their active substances, such as phenolic compounds which are part of the essential oils, as well as in tannin (Betoni *et al*. 2006). The antimicrobial properties of various plants have been investigated by a number of researchers (Kloucek *et al.* 2006; Molina-Salinas *et al*. 2006; Weckessera *et al*. 2006; Al Fatimi *et al.* 2007; Fazlara *et al.* 2007). However, few studies on synergism have been carried out (Kuzma *et al*. 2005; Betoni *et al*. 2006; Nascimento *et al*. 2006; Cruz *et al.* 2007; du Toit *et al.* 2007; Shokeen *et al.* 2008). In this study, the antimicrobial activity and synergistic effects of different parts of five local plants extracts were utilized and evaluated against *E. coli* and *S. aureus* strains.

MATERIALS AND METHODS

Materials

The following plant materials were used in this study (**Table 1**): *Cakile maritima* (roots and shoots), *Cakile maritima* (seeds), *Mesembryanthemum crystallinum* (whole plant), *Atriplex halimus* (leaves), *Withania somnifera* (leaves) which had been collected from different areas of the Mediterranean Sea Beach. *Marrubium vulgare* (stem and leaves) was collected from Alzawaida fields, Gaza Strip, Palestine.

The microorganisms which have been used in this study are *E. coli* and *S. aureus* strains which were isolated from clinical samples delivered from El-Shifa Hospital, Microbiology Department and identified using morphological and biochemical diagnostic tests at the Medical Technology Department at the Islamic University of Gaza.

Culture media

The following culture media Brain Heart Infusion Broth (HiMedia, India) and Mueller-Hinton agar (HiMedia) were used in the well diffusion method and disk diffusion methods.

Received: 1 February, 2010. Accepted: 20 October, 2010.

Original Research Paper

Table 1 Ethnobotanical data of the investigated plants in this study.

Scientific name (family)	Plant origin	Solvent	Antimicrobial or medicinal activity	References
Cakile maritime (*Brassicaceae*)	Leaves	Methanol and chloroform	*S. aureus*, *Micrococcus luteus*, *Bacillus cereus*, *Salmonella enterica*, *P. aeruginosa*, *P. fluorescens*, *P. marginalis*, *E. coli*, *Candida albicans*	Meot-Duros *et al*. 2008
Withania somnifera (*Solanaceae*)	Roots	70% methanol	Immunostimulatory action, antitumour activity	Davis and Kuttan 1998
	Roots	Methanol	Immunostimulatory action, *Listeria monocytogenes*	Teixeira *et al*. 2006
	Roots	Alcoholic	Antiperoxidative action	Charasia *et al*. 2000
	Leaves	Methanol and hexane	*S. typhimurium*, *E. coli*	Arora *et al*. 2004
	Roots	Methanol and hexane	Synergistic effect with *Tibrium* against *S. typhimurium* and *E. coli*	Arora *et al.* 2004
	Roots and leaves	Methanol and water	*E. coli*, *S. aureus*, *S. typhimurium*	Owais *et al*. 2005
Marrubium vulgare (*Labiatae*)	Leaves and stems	Water, hexane, acetone and methanol	*Mycobacterium tuberculosis*	Molina-Salinas *et al.* 2006
	Leaves and flowers	Ethanol	*Bacillus subtilis*	Al-Bakri and Afifi 2007
	leaves	Methanol	Hypoglycemic agent, Antilipemic agent	Herrera-Arellano *et al.* 2004
Atriplex halimus (*Amaranthaceae*)	Leaves and stems	Methanol	Antioxidant activity	Benhammou *et al*. 2008
Mesembryanthemum crystallinum (*Aizoaceae*)	Shoots	Methanol	Antioxidant activity	Hanen *et al*. 2009

Preparation of plant extract

The plant materials were dried and pulverized into fine powder. All plant parts were extracted using four different methods (except *C. maritima* (seeds) which was not extracted by water reflux), as follows:

1. Water reflux

Each 2.5 g powdered plant material was extracted by refluxing with 25 ml distilled water for 30 min and was kept overnight at room temperature before filtration. After filtration, the volume of the extracts were concentrated by evaporation at room temperature until the volume of the crude extract became around 4-5 ml (Al-Bakri *et al*. 2007).

2. Ethanol

3 g of powdered plant materials were extracted with 30 ml of 80% (v/v) ethanol at room temperature for 8 h and then was filtered and concentrated by evaporation at room temperature until the volume of each extract became 4-5 ml (Al-Bakri *et al*. 2007).

3. Methanol

5 g of powdered plant materials was finely macerated at room temperature in 150 ml of 80% (v/v) methanol (Merck, Darmstadt, Germany) for 5 days which was then filtered and concentrated as mentioned above (Cown 1999).

4. Ethanol reflux

Each 2.5 g powdered plant material was extracted by refluxing with 25 ml ethanol for 30 min and was kept overnight at room temperature before filtration. The volumes of the extracts were concentrated as mentioned above to a volume of 4-5 ml (Al-Bakri *et al*. 2007).

Antibacterial activity test

Antibacterial activity was determined by the well diffusion method according to NCCLS (1993). The bacterial cultures were grown in Brain Heart Infusion liquid medium at 37°C. After 4 h of growth, each microorganism was inoculated by streaking the swab over the entire surface of Mueller Hinton agar plates. After allowing the inocula to dry at room temperature, 6 mm-diameter wells were bored in the agar. Each extract was checked for antibacterial activity by introducing 10 µl into the well. The plates were allowed to stand at room temperature for 1 h for extract to diffuse into the agar and then they were incubated at 37°C for 18 h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the well.

Bacterial strain resistance

This was carried out by using disk diffusion technique (Bauer *et al*. 1966). The method measures microbial growth inhibition at the surface of an inoculated medium around paper discs of various antibiotics (Merck) as shown in **Table 2**.

Incubation was performed at 37°C for 18 h. The assessment of bacterial resistance was based on the measurement of the diameter of the inhibition zone formed around the antibiotic disc.

Evaluation of the synergistic effect

The bacterial cultures were grown in Brain Heart liquid medium at 37°C. After 4 h of growth, each microorganism at a concentration of 10^6 cells/mL was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, the antibiotic filter paper discs of 6 mm in diameter saturated with 10 µL plant extract were placed on the surface of each inoculated plate. The plates were incubated at 37°C for 24 h. The diameters of cleared zones were measured and compared with that of the antibiotic alone (Betoni *et al.* 2006).

RESULTS AND DISCUSSION

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. For the last few years many natural antimicrobial products have been isolated from a wide range of

Table 2 List of antibiotics potency.

Antibiotic	Antibiotic potency (µg)
Amikacin	30
Amixicillin	30
Amoxycalv	30
Minocyclin	30
Kanamycin	24
Tetracycline	30
Tobramycin	10
Streptomycin	10
Erythromycin	15
Vancomycin	30
Cefuroxim	30
Ceftazidim	30
Clindamicin	10

Table 3 Antimicrobial and synergistic effect of some plant tissue extracts, extracted by water reflux on *E. coli* (all values in mm).

Antibiotic	Antibiotic alone	*C. maritima* (Root + shoot)		*M. crystallinum* (whole plant)		*A. halimus* (leaves)		*W. somnifera* (leaves)		*M. vulgare* (stem)		*M. vulgare* (leaves)	
		Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti
Amoxyclav	-		-		-		-		-		-		-
Minocyclin	-		-		-		-		-		-		-
Amikacin	22		22		20		-		-		-		-
Tetracycline	-	No	-	No	-	No	-	No	-	No	-	No	-
Kanamycin	9	effect	-	effect	-	effect	-	effect	-	effect	-	effect	-
Topramycin	-		-		-		-		-		-		-
Cefuroxim	-		-		-		-		-		-		-
Ceftazidim	-		-		-		-		-		-		-

(-) no inhibition zone, Anti: antibiotic, Ex: extract, disc diameter (6 mm), well diameter (7 mm). *C. maritima*: *Cakile maritima*, *M. crystallinum*: *Mesembryanthemum crystallinum*, *A. halimus*: *Atriplex halimus*, *W. somnifera*: *Withania somnifera*, *M. vulgare*: *Marrubium vulgare.*

animal, plant and bacterial species. These compounds, which comprise a diverse class of molecules used in natural host defence, may have therapeutic potential in the treatment of infections in humans and are now considered to be an alternative way for future therapy (Nascimento *et al*. 2000; Akinsulire *et al.* 2007). The aim of this study was the evaluation and comparison of the antimicrobial activity and synergistic effects of different parts of five local plants extracts against *E. coli* and *S. aureus* strains.

Our results show that there was no antibacterial activity in the plant extracts against *E. coli* except from *C. maritima* seeds (extracted using ethanol for 8 h) with a zone of inhibition = 13 mm (**Table 3**). The extracts from *W. somnifera*, *M. vulgare* stems and *M. vulgare* (extracted by ethanol for 8 h) were the most active compounds against *S. aureus* with respect to the inhibition panel. Antibacterial activity against *S. aureus* was measured from the extracts of *W. somnifera* left with an inhibition zone = 25 mm, *M. vulgare* stems with an inhibition zone = 15 mm and *M. vulgare* with an inhibition zone = 13 mm.

Our results agree with other observations that the susceptibility of bacteria to *W. somnifera* was highly observed (Davis and Kuttan 1998; Charasia *et al*. 2000; Arora *et al.* 2004; Owais *et al*. 2005; Teixeira *et al.* 2006). The extracts as well as different isolated bioactive constituents of *W. somnifera* have been reported to possess adaptogenic, anticancer, anti-convulsant, immunomodulatory, antioxidative and neurological effects. The plant is also considered efficacious in the treatment of arthritis, geriatric, behavioural and stress related problems (Gupta and Rana 2007). Administration of *W. somnifera* extract significantly reduced the leucopenia induced by sublethal dose of cyclophosphamide in mice which indicate that *W. somnifera* could reduce the cyclophosphamide induced toxicity and its usefulness in cancer therapy (Davis and Kuttan 1998) (**Table 1**).

W. somnifera leaves extract protects mice from a lethal dose of *Listeria monocytogenes* when administered prophylactically at 100, 250 and 500 mg/kg for 10 days, with survival rates up to 30%. These doses also prevented the myelosuppression and the splenomegaly caused by a sublethal infection with L. monocytogenes, due to increased numbers of granulocyte–macrophage progenitors (CFU-GM) in the bone marrow (Silvia *et al.* 2006; RajaSankar *et al*. 2009) (**Table 1**).

A. halimus produce the polyphenols and other bioactive substances potentially useful for medicinal properties and as natural food preservation. Over all *A. halimus* possess potential antioxidant activity. With relation to *M. vulgare*, it has been reported that leaf extracts possess vasorelaxant, antioxidative, antiinflammatory, hypotensive, antispasmodic, antinociceptive, antilipemic and hypoglycaemic effects (Herrera-Arellan *et al.* 2004) (**Table 1**). We then proceeded to measure the synergistic inhibition effect against *E. coli* and *S. aureus* using these plants extracts.

Evaluation of synergistic effect

Even though pharmaceutical industries have produced a number of new antimicrobial drugs in the last years, resistance to these drugs by microorganisms has increased which can be explained by the genetic ability of bacteria to transmit and acquire resistance to drugs used as therapeutic agents (Nascimento *et al*. 2000). Therefore new drugs should be produced continuously.

Few studies have been reported on synergism (Nascimento *et al*. 2000; Aburjai *et al.* 2001; Aqil *et al.* 2005; Betoni *et al*. 2006). Asynergistic increase in the antibacterial effect of Tibrim was noticed when MIC of Tibrim was supplemented with *W. somnifera* extracts (Arora *et al*. 2004).

Thus, in our study, we evaluated *in vitro* synergism between extracts of (i.e. *C. maritima* (root and shoot), *M. crystallinum* (whole plant), *M. vulgare* (stems), *M. vulgare* (leaves) and *C. maritima* (root) and antimicrobial drugs utilized against *E. coli* and *S. aureus* strains by using the disc diffusion method. The seven plant extracts tested showed various degrees of synergistic inhibition effect against the two bacterial strains investigated as presented in **Tables 3-10**.

1. Against Escherichia coli

The plant extracts differed significantly in their synergistic ability to inhibit the growth of *E. coli* depending on the method of extraction. There was no synergistic effect of any plant extracts against *E. coli,* which was extracted using water reflux method (**Table 3**).

Table 4 summarizes the synergistic effect of plant extracts against *E. coli*, which was extracted using ethanol for 8 h. Among the seven extracts (i.e. *C. maritima* (root and shoot), *M. crystallinum* (whole plant), *M. vulgare* (stems), *M. vulgare* (leaves) *A. halimus* (leaves) and *C. maritima* (root and shoot) added as crude extract of 10 µl/well, *M. vulgare* (stems and leaves) had the most synergistic inhibitory effect against *E. coli.*

The results showed that *M. vulgare* (leaves and stem) had a synergistic effect with various antibiotics and was able to suppress the *E. coli* growth and their extracts alone were ineffective.

Table 5 summarizes the results obtained with plant extracts which had been extracted by methanol 5 days. All extracts by methanol reflux had no synergistic effect against *E. coli.*

Table 6 summarizes the results obtained with plant extracts which had been extracted by ethanol reflux. All extracts by ethanol reflux had no synergistic effect against *E. coli* except *M. vulgare* (leaves) extracts, with a synergistic effect with amikacine and kanamycin only.

The ethanol 8 hours extract of different parts of the five local plants against *E. coli* exerted greater synergistic activity than corresponding either methanol or water reflux extracts (**Table 3-6**) at the same concentrations. These results

Table 4 Antimicrobial and synergistic effect of some plant tissue extracts, extracted by ethanol for 8 hrs on *E. coli* (all values in mm).

Antibiotic	Antibiotic alone	*C. maritima* (Root + shoot)		*C. maritima* (seed)		*M. crystallinum* (whole plant)		*A. halimus* (leaves)		*W. somnifera* (leaves)		*M. vulgare* (stem)		*M. vulgare* (leaves)	
		Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti
Amoxyclav	-		8		-		-		-		-		-		-
Minocyclin	-		12		10		-		-		-		-		10
Amikacin	22		23		20		-		22		22		23		22
Tetracyclin	-	No	10	13	9	No	13	No	-	No	-	No	-	No	7
Kanamycin	9	effect	11		8	effect	-	effect	-	effect	-	effect	-	effect	9
Topramycin	-		10		10		-		-		-		7		-
Cefuroxim	-		-		-		-		-		-		-		9
Ceftazidim	-		-		8		-		-		-		-		10

(-) no inhibition zone, Anti: antibiotic, Ex: extract, disc diameter (6 mm), well diameter (7 mm). *C. maritima*: *Cakile maritima*, *M. crystallinum*: *Mesembryanthemum crystallinum*, *A. halimus*: *Atriplex halimus*, *W. somnifera*: *Withania somnifera*, *M. vulgare*: *Marrubium vulgare.*

Table 5 Antimicrobial and synergistic effect of some plant tissue extracts, extracted by Methanol for 5 days on *E .coli* (all values in mm).

Antibiotic	Antibiotic alone	*C. maritima* (Root + shoot)		*C. maritima* (seed)		*M. crystallinum* (whole plant)		*A. halimus* (leaves)		*W. somnifera* (leaves)		*M. vulgare* (stem)		*M. vulgare* (leaves)	
		Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti
Amoxyclav	-		-		-		-		-		-		-		-
Minocyclin	-		-		-		-		-		-		-		-
Amikacin	22		21		20		23		22		-		-		-
Tetracycline	-	No	-	No	-	No	-	No	-	No	-	No	-	No	-
Kanamycin	9	effect	-	effect	-	effect	-	effect	-	effect	-	effect	-	effect	-
Topramycin	-		-		-		-		-		-		-		-
Cefuroxim	-		-		-		-		-		-		-		-
Ceftazidim	-		-		-		-		-		-		-		-

(-) no inhibition zone, Anti: antibiotic, Ex: extract, disc diameter (6 mm), well diameter (7 mm). *C. maritima*: *Cakile maritima*, *M. crystallinum*: *Mesembryanthemum crystallinum*, *A. halimus*: *Atriplex halimus*, *W. somnifera*: *Withania somnifera*, *M. vulgare*: *Marrubium vulgare.*

Table 6 Antimicrobial and synergistic effect of some plant tissue extracts, extracted by ethanol reflux on *E. coli* (all values in mm).

Antibiotic	Antibiotic alone	*C. maritima* (Root + shoot)		*C. maritima* (seed)		*M. crystallinum* (whole plant)		*A. halimus* (leaves)		*W. somnifera* (leaves)		*M. vulgare* (stem)		*M. vulgare* (leaves)	
		Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti
Amoxyclav	-		-		-		-		-		-		-		-
Minocyclin	-		-		-		-		-		-		-		-
Amikacin	22		21		20		19		-		-		-		27
Tetracyclin	-	No	-	No	-	No	-	No	-	No	-	No	-	No	-
Kanamycin	9	effect	-	effect	-	effect	-	effect	-	effect	-	effect	-	effect	12
Topramycin	-		-		-		-		-		-		-		-
Cefuroxim	-		-		-		-		-		-		-		-
Ceftazidim	-		-		-		-		-		-		-		-

(-) no inhibition zone, Anti: antibiotic, Ex: extract, disc diameter (6 mm), well diameter (7 mm). *C. maritima*: *Cakile maritima*, *M. crystallinum*: *Mesembryanthemum crystallinum*, *A. halimus*: *Atriplex halimus*, *W. somnifera*: *Withania somnifera*, *M. vulgare*: *Marrubium vulgare.*

confirmed the evidence in previous studies reported that ethanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents, such as water and methanol (Cowan 1999; Blatnik and Lesnicar 2006).

2. Against Staphylococcus aureus

Although the antimicrobial activities of *C. maritima* (roots and shoots), *C. maritima* (seeds), *M. crystallinum* (whole plant), *A. halimus* (leaves) and *W. somnifera* (leaves) extracts have not been relatively high, synergism assays were carried out for them and the synergism rate differed significantly in their synergistic ability to inhibit the growth of *S. aureus* depending on the method of extraction and the plant extracts. The synergistic capacity against *S. aureus* was promising for the extracts of some plants such as *M. vulgare* (stems and leaves) and *M. crystallinum* (whole plant) which had the most synergistic inhibitory effect against *S. aureus* which presented synergism with most drugs. *M. crystallinum* is well known for its enzymatic antioxidant activity engaged in detoxification of reactive oxygen species (ROS) (**Table 1**) (lesak and Miszalski 2003). Moreover, similar antibacterial results were observed with *C. maritimum* which inhibited the growth of *Bacillus cereus* and *Micrococcus luteus* (**Table 1**) (Meot-Duros *et al*. 2007).

Table 7 summarizes the synergistic effects of plant extracts against *S. aureus* which were extracted by water reflux. Among the seven extracts (i.e., *C. maritima* (root and shoot), *M. crystallinum* (whole plant), *M. vulgare* (stems), *M. vulgare* (leaves) and *C. maritima* (root and shoot) were added as a crude extract of 10 µl/well. *M. vulgare* (stems and leaves) and *M. crystallinum* (whole plant) had the most synergistic inhibitory effect against *S. aureus.*

Our results also showed that *M. vulgare* (leaves and stem) had a synergistic effect with various antibiotics and was able to suppress the *S. aureus* growth while their extracts alone were ineffective. The highest synergistic effect was observed with tetracycline and minocyclin. *C. maritima* (root and shoot) extract, also had a synergistic effect with minocyclin, topramycin, amoxicillin, erythromycin and vancomycin. *W. somnifera* (leaves) extract had synergistic effect only with kanamycin and topramycin. *M. vulgare* (leaves) extract had a synergistic effect with minocyclin, tetracyclin, kanamycin, topramycin, erythromycin, streptomycin and vancomycin, however the highest synergistic effect was observed with minocyclin. The results showed that *A. halimus* (leaves) extract which was extracted by water reflux method had no synergistic effect with all antibiotic*s* tested in our study (**Table 7**).

Table 7 Antimicrobial and synergistic effect of some plant tissue extracts, extracted by water reflux on *S. aureus* (all values in mm).

Antibiotic	Antibiotic alone	*C. maritima* (Root + shoot)		*M. crystallinum* (whole plant)		*A. halimus* (leaves)		*W. somnifera* (leaves)		*M. vulgare* (stem)		*M. vulgare* (leaves)	
		Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti
Minocyclin	34		38		46		-		-		40		41
Tetracycline	32		44		46		-		-		46		35
Kanamycin	20		20		26		-		28		26		32
Topramycin	24	No effect	25	No effect	26	No effect	-	No effect	28	No effect	25	No effect	28
Amoxicillin	19		22		23		-		-		-		-
Clindamicin	20		13		-		-		-		-		-
Erythromycin	20		28		35		-		-		24		30
Streptomycin	-		-		-		-		-		-		12
Vancomycin	18		25		25		-		-		22		20

(-) no inhibition zone, Anti: antibiotic, Ex: extract, disc diameter (6 mm), well diameter (7 mm). *C. maritima*: *Cakile maritima*, *M. crystallinum*: *Mesembryanthemum crystallinum*, *A. halimus*: *Atriplex halimus*, *W. somnifera*: *Withania somnifera*, *M. vulgare*: *Marrubium vulgare.*

Table 8 Antimicrobial and synergistic effect of some plant tissue extracts, extracted by ethanol for 8 hrs on *S. aureus* (all values in mm).

Antibiotic	Antibiotic alone	*C. maritima* (Root + shoot)		*C. maritima* (seed)		*M. crystallinum* (whole plant)		*A. halimus* (leaves)		*W. somnifera* (leaves)		*M. vulgare* (stem)		*M. vulgare* (leaves)	
		Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti
Minocyclin	34		33		54		42		46		40		24		39
Tetracycline	32		43		42		34		-		48		35		38
Kanamycin	20		28		36		22		22		32		22		24
Topramycin	24	No effect	30	No effect	30	No effect	26	No effect	30		30		-		30
Amoxicillin	19		29		-		25		26	25	14	15	22	13	20
Clindamicin	20		28		-		18		-		12		17		22
Erythromycin	20		27		30		30		28		24		21		20
Streptomycin	-		-		-		-		-		12		-		20
Vancomycin	18		25		27		30		28		24		23		25

(-) no inhibition zone, Anti: antibiotic, Ex: extract, disc diameter (6 mm), well diameter (7 mm). *C. maritima*: *Cakile maritima*, *M. crystallinum*: *Mesembryanthemum crystallinum*, *A. halimus*: *Atriplex halimus*, *W. somnifera*: *Withania somnifera*, *M. vulgare*: *Marrubium vulgare.*

Table 9 Antimicrobial and synergistic effect of some plant tissue extracts, extracted by methanol for 5 days on *S. aureus* (all values in mm).

Antibiotic	Antibiotic alone	*C. maritima* (Root + shoot)		*C. maritima* (seed)		*M. crystallinum* (whole plant)		*A. halimus* (leaves)		*W. somnifera* (leaves)		*M. vulgare* (stem)		*M. vulgare* (leaves)	
		Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti
Minocyclin	34		33		-		40		48		-		-		-
Tetracycline	32		35		-		44		-		-		-		-
Kanamycin	20		40		-		32		22		-				-
Topramycin	24	No effect	28	No effect	-	No effect	26	No effect	24	No effect	-	No effect	18	No effect	-
Amoxicillin	19		30		-		10		26		-		-		-
Clindamicin	20		11		-		-		-		-		-		17
Erythromycin	20		28		-		-		25		-		-		30
Streptomycin	-		-		-		-		-		-		-		-
Vancomycin	18		-		-		-		30		-		-		29

(-) no inhibition zone, Anti: antibiotic, Ex: extract, disc diameter (6 mm), well diameter (7 mm). *C. maritima*: *Cakile maritima*, *M. crystallinum*: *Mesembryanthemum crystallinum*, *A. halimus*: *Atriplex halimus*, *W. somnifera*: *Withania somnifera*, *M. vulgare*: *Marrubium vulgare.*

Table 8 lists the synergistic effects of all plant extracts against *S. aureus* which were extracted by ethanol for 8 h. The seven plant extracts (i.e. *C. maritima* (root and shoot), *M. crystallinum* (whole plant), *M. vulgare* (stems), *M. vulgare* (leaves), *A. halimus* (leaves), *W. somnifera* (leaves) and *C. maritime* (root and shoot) had a synergistic inhibitory effect against *S. aureus* with most antibiotics as shown in **Table 8**. The highest synergistic effect was observed with tetracycline and minocyclin.

Table 9 summarizes the synergistic effects of plant extracts which had been extracted by methanol for 5 days. All these extracts had a synergistic inhibitory effect against *S. aureus* with various antibiotics as shown in **Table 9**. The highest synergistic effect was observed with tetracycline and minocyclin.

On the other hand, the extracts from *C. maritima* seeds, *W. somnifera* leaves and *M. vulgare* stems had no synergistic effect with any antibiotic used against *S. aureus.*

Table 10 represents the synergistic effect of the different plant extracts which were extracted by ethanol reflux against *S. aureus.*

All these extracts had a synergistic inhibitory effect against *S. aureus* with various antibiotics as shown in **Table 10**. The highest synergistic effect was observed with tetracycline, minocyclin and amoxicillin.

Among the protein synthesis inhibitors, tetracycline showed the highest rate of synergism with all the extracts, followed by minocycline and amoxacillin, minocycline is a second-generation of tetracycline that effectively crosses the blood–brain barrier and considered as bacterial protein synthesis inhibitors. The synergistic capacity against *S. aureus* was promising for the extracts of all plants which presented synergism with the most tested drugs.

In general, the plant antibiotic substances and their synergistic effect appear to be an inhibitor to *S. aureus* (the Gram-positive organism) rather than to *E. coli* (the Gram-negative type) which could be due to the difference in the structure of the cell wall. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria (Burn 1988). Therefore, access of most compounds to the peptidoglycan layer of the cell wall is hindered by the outer lipopolysaccharide layer.

Table 10 Antimicrobial and synergistic effect of some plant tissue extracts, extracted by ethanol reflux on *S. aureus* (all values in mm).

Antibiotic	Antibiotic alone	*C. maritima* (Root + shoot)		*C. maritima* (seed)		*M. crystallinum* (whole plant)		*A. halimus* (leaves)		*W. somnifera* (leaves)		*M. vulgare* (stem)		*M. vulgare* (leaves)	
		Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti	Extract alone	Ex +Anti
Minocyclin	34		30		42		40		45		48		50		44
Tetracycline	32		34		44		44		34		54		68		36
Kanamycin	20		25		30		24		28		32		42		18
Topramycin	24	No effect	31	No effect	28	No effect	25	No effect	28	No effect	30	No effect	28	No effect	30
Amoxicillin	19		42		12		22		34		-		10		34
Clindamicin	20		32		20		17		-		13		13		10
Erythromycin	20		30		30		26		29		27		24		28
Streptomycin	-		16		-		15		-		-		10		-
Vancomycin	18		25		27		25		21		20		21		25

(-) no inhibition zone, Anti: antibiotic, Ex: extract, disc diameter (6 mm), well diameter (7 mm). *C. maritima*: *Cakile maritima*, *M. crystallinum*: *Mesembryanthemum crystallinum*, *A. halimus*: *Atriplex halimus*, *W. somnifera*: *Withania somnifera*, *M. vulgare*: *Marrubium vulgare.*

CONCLUSION

Our finding express:

1. Plant extracts have a potential antimicrobial effect as compared to commercial antimicrobial compounds against microorganisms of *E. coli* and *S. aureus* which can be used in the treatment of infectious diseases caused by resistant *E. coli* and *S. aureus*.
2. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.
3. Our study provides a new rationale and added inputs for the use of plant extracts and synergism effect against the most common pathogenic microorganisms such as *E. coli* and *S. aureus*.

REFERENCES

Aboaba O, Smith S, Olude F (2006) Antibacterial effect of edible plant extract on *Escherichia coli* 0157:H7. *Pakistan Journal of Nutrition* **5**, 325-327

Aburjal T, Darwish R, Al-Khalil S, Mahgzah A, Al-Abbdi A (2001) Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Pseudomonas aeruginosa*. *Journal of Ethnopharmacology* **76**, 39-44

Al-Bakri A, Fatma U, Afifi F (2007) Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration. *Journal of Microbiological Methods* **68**, 19-25

Al-Fatimi M, Wurster M, Schroder G, Lindequist U (2007) Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *Journal of Ethnopharmacology* **111**, 657-666

Akinsulire OR, Ibukun EA, Tayo A, Toyin A, Tolu O (2007) *In vitro* antimicrobial activity of crude extracts from plants *Bryophyllum Pinnatum* and *Kalanchoe Crenata. African Journal of Traditional, Complementary, and Alternative Medicines* **4**, 338-344

Aqil F, Khan M, Owais M, Ahmad I (2005) Effect of certain bioactive plant extracts on clinical isolates of β-lactamase producing methicillin resistant *Staphylococcus aureus*. *Journal of Basic Microbiology* **45**, 106-114

Arora S, Dhillon S, Rani G, Nagpal A (2004) The *in vitro* antibacterial/synergistic activities of *Withania somnifera* extracts. *Fitoterapia* **75**, 385-388

Bassam A, Ghaleb A, Naser J, Awni A, Kamel A (2006) Antibacterial activity of four plant extracts used in Palestine in Folkloric medicine against methicillin-resistant *Staphylococcus aureus*. *Turkish Journal of Biology* **30**, 195-198

Bauer A W, Kirby M M, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* **45**, 493-496

Betoni J, Mantovani R, Barbosa L, di Stasi L Júnior A (2006) Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Memórias do Instituto Oswaldo Cruz, Rio de Janeiro* **101**, 387-390

Blatnik J, Lesnicar G (2006) Propagation of methicillin-resistant *Staphylococcus aureus* due to the overloading of medical nurses in intensive care units. *The Journal of Hospital Infection* **63**, 162-166

Burn P (1988) Amphitropic proteins: A new class of membrane proteins. *Trends in Biochemical Sciences* **13**, 79-83

Cowan MM (1999) Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12**, 564-582

Cruz S, Santos O, Barbosa A, de Mélo L, Alviano S, Antoniolli R, Alviano S, Trindade C (2007) Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. *Journal of Ethnopharmacology* **111**, 409-412

Davis L, Kuttan G (1998) Suppressive effect of cyclophosphamide-induced toxicity by *Withania somnifera* extract in mice. *Journal of Ethnopharmacology* **62**, 209-214

Deans G, Ritchie A (1987) Antimicrobial properties of plant essential oils. *International Journal of Food Microbiology* **5**, 165-180

du Toit K, Elgorashi E, Malan F, Mulholland A, Drewes E, van Staden J (2007) Antibacterial activity and QSAR of homoisoflavanones isolated from six Hyacinthaceae species. *South African Journal of Botany* **73**, 236-241

Falleh H, Ksouri R, Oueslati S, Guyot S, Magné C, Abdelly C (2009) Interspecific variability of antioxidant activities and phenolic composition in *Mesembryanthemum* genus. *Food and Chemical Toxicology* **47**, 2308-2313

Fazlara A, Najafzadeh H, Lak E (2008) The potential application of plant essential oils as natural preservatives against *Escherichia coli* O157: H7. *Pakistan Journal of Biological Science* **11**, 2054-2061

Herrera-Arellano A, Aguilar-Santamaría L, García-Hernández B, Nicasio-Torres P, Tortoriello J (2004) Clinical trial of *Cecropia obtusifolia* and *Marrubium vulgare* leaf extracts on blood glucose and serum lipids in type 2 diabetics. *Phytomedicine* **11**, 561-566

Kloucek P, Svobodova B, Polesny Z, Langrova I, Smrcek S, Kokoska L (2006) Antimicrobial activity of some medicinal barks used in Peruvian Amazon. *Journal of Ethnopharmacology* **111**, 427-429

Kumar K, Devi SS, Krishnamurthi K, Kanade GS, Chakrabarti T (2007) Enrichment and isolation of endosulfan degrading and detoxifying bacteria. *Chemosphere* **68**, 317-22

Kuźmaa Ł, Żalskib M, Walenckac E, Żalska B, Wysokin H (2007) Antimicrobial activity of diterpenoids from hairy roots of *Salvia sclareal*. *Phytomedicine* **14**, 31-35

Lesak S, Miszalski I (2003) Superoxide dismutase-like protein from roots of the intermediate C3-CAM plant *Mesembryanthemum crystallinum* L. in *in vitro* culture. *Plant Science* **164**, 497-505

Lesse AJ (1995) Penicillins and other cell wall active agents. In: *Essentials of Pharmacology*, WB Sanders Co., Philadelphia, pp 362-373

Meot-Duros L, le Floch G, Magné C (2007) Radical scavenging, antioxidant and antimicrobial activities of halophytic species. *Journal of Ethnopharmacology* **116**, 258-262

Nascimento G, Locatelli J, Freitas P, Silva G (2000) Antibacterial activity of plant extracts and phytochmicals on antibiotic resistant bacteria. *Brazilian Journal of Microbiology* **31**, 247-256

NCCLS (1993) Peformance standards for antimicrobial disc suspectibility tests. Approved standard NCCLS publication M2-A5, Villanova, PA, USA

Owen R, Palombo E (2007) Anti-listerial activity of ethanolic extracts of medicinal plants, *Eremophila alternifolia* and *Eremophila duttonii*, in food homogenates and milk. *Food Control* **18**, 387-390

Perumal R (2005) Antimicrobial activity of some medicinal plant from India. *Fitoterapia* **76**, 697-699

Prashanth K, Neelam S, Harish P, Rajani M (2006) Search for antibacterial and antifungal agents from selected Indian medicinal plants. *Journal of Ethnopharmacology* **107**, 182-188

RajaSankar S, Manivasagam T, Sankar V, Prakash S, Muthusamy R, Krishnamurti A, Sankar S (2009) *Withania somnifera* root extract improves catecholamines and physiological abnormalities seen in a Parkinson's disease model mouse. *Journal of Ethnopharmacology* **125**, 369-373

Shokeen P, Bala M, Tandon V (2008) Evaluation of the activity of 16 medicinal plants against *Neisseria gonorrhoeae*. *International Journal of Antimicrobial Agents* **33**, 86-91

Weckessera S, Engela K, Simon-Haarhausa B, Wittmerb A, Pelzb K, Schempp C (2006) Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. *Phytomedicine* **14**, 508-51