

## Synergistic effect of *Thymbra spicata* L. extracts with antibiotics against multidrug-resistant *Staphylococcus aureus* and *Klebsiella pneumoniae* strains

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### ABSTRACT

**Objective(s):** To evaluate the *in vitro* interaction between different extracts of *Thymbra spicata* L. and certain antimicrobial drugs of different mechanisms, including ampicillin, cefotaxime, amikacin and ciprofloxacin. This study was performed against multidrug-resistant strains of *Staphylococcus aureus* and *Klebsiella pneumoniae*.

**Materials and Methods:** Evaluation of antibacterial activity and synergy interaction between plant extracts and antimicrobial agents was carried out using checkerboard microdilution.

**Results:** Different interactions (synergistic, additive and indifference) were observed between plant crude extracts and used antibiotics depending on the strain. The fractional inhibitory concentration (FIC) index ranged from 0.02 to 1.5 for *S. aureus* and 0.25 to 2 for *K. pneumoniae* strains. The best synergistic capacity appeared with cefotaxime against *S. aureus* strains, where the activity of cefotaxime was increased from 8- to 128-fold.

**Conclusion:** These results may indicate that *T. spicata* extracts potentiates the antimicrobial action of antibiotics, suggesting a possible utilization of this herb in combination therapy against emerging multidrug-resistance *S. aureus* and *K. pneumoniae*.

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### Introduction

In recent years, the Infectious Diseases Society of America has highlighted a group of pathogens ESKAPE that they currently cause the majority of hospital infections and can effectively "escape" the biocidal action of antibiotics. *Staphylococcus aureus* and *Klebsiella pneumoniae* are members in this group which includes *Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species (1). The steadily increasing in multidrug-resistant bacteria to existing antibiotics is a serious problem that significantly causing treatment failure of infections and increase mortality rates (2, 3). There is an urgent need to develop new antibacterial substances or new compounds that block resistance mechanisms and improve treatment to eradicate these resistant strains. Treatment with antibacterial combinations, using two or more antibacterial agents is one of the most important strategies to overcome multidrug-resistant organism (4).

Recently, plant antimicrobials have been found to be synergistic enhancers in that though they may not

have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug (5-8). There are some generally accepted mechanisms of this interaction, including inhibition of protective enzymes, combination of membrane active agents, sequential inhibition of common biochemical pathways, and the use of membranotropic agents to enhance the diffusion of other antimicrobials (9).

Phytotherapy has many potentially significant advantages associated with the synergistic interactions like, increased efficiency, reduction of undesirable effects, increase in the stability or bioavailability of the free agents and obtaining an adequate therapeutic effect with relatively small doses, when compared with a synthetic medication (10).

During the past ten years, several reviews substantiated the effectiveness of combinations of plants with conventional antimicrobials (10-12). However, no studies were found that investigated the effect of combination of *Thymbra spicata* extract with antibiotics against multidrug (MDR) *S. aureus*

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and *K. pneumoniae*. *T. spicata*, (Lamiaceae), is a native plant in the flora of Syria (13). It is an evergreen perennial Shrub that tends to grow to 0.5 m on dry, sunny hillsides and high dry meadows (14). It is a well-known medicinal plant that used in folk medicine traditions. The essential oil found in different parts of *T. spicata* makes it an important antibacterial and antioxidant natural source. The infusion of this plant is used for treating of respiratory and sore throat infection. Besides, it used as a spice that gives a good flavor and taste to meals (15).

It is well known that antimicrobial activities of plant extract against tested bacteria differed, depending on location (16). So, the aim of the present study was to examine antibacterial effect of different *T. spicata* extract against multidrug-resistant strains of *S. aureus* and *K. pneumoniae* and to investigate the synergy between these extracts and commonly used antibiotics.

## Materials and Methods

### Bacterial strains

Twelve different strains of *S. aureus* and *K. pneumoniae* were used to evaluate the synergetic activity of plant extracts with antibiotic including, two reference strains *S. aureus* ATCC 25923 and *K. pneumoniae* ATCC 700603. The other ten strains were clinical isolates that obtained from Aleppo University Hospital containing one sensitive strain and four multidrug-resistant for each species. Bacterial suspension was made from fresh culture and stored at  $-20^{\circ}\text{C}$  using 30% glycerol.

### Antimicrobial agents

Four antibiotics were used for synergism assays. These include: ampicillin, cefotaxime, amikacin and ciprofloxacin. All these antibiotics were obtained from Asia Pharmaceutical Co except ciprofloxacin, which were obtained from Obari Pharmaceutical Co.

### Preparation of plant extracts

Collected wild plant materials were washed with sterile water and allowed to drain and dried at  $25-30^{\circ}\text{C}$  in a place not exposed to sunlight and without applying any heat treatment to reduce the loss active components. Then the dried leaves were crushed to powder and kept in refrigerator at  $4^{\circ}\text{C}$  until use. Dried, ground leaves (20 g for each extract) were extracted with 100 ml water, ethanol and petroleum ether by maceration. The extracts were filtered through a Buchner funnel with Whatman filter paper number 1. After filtration, extracts were evaporated under reducing pressure to dryness at  $45^{\circ}\text{C}$  on a rota-evaporator (B.U.C.H.I). The collected crude extracts were stored at  $4^{\circ}\text{C}$  until using. All extracts were redissolved in dimethyl sulfoxide (DMSO 1%) with the exception of the aqueous extracts which

redissolved in distilled water to 200 mg/ml. The reconstituted extract solution was sterilized by filtering through  $0.45\ \mu\text{m}$  membrane filter before using in bioassay (17).

### Determination of minimum inhibitory concentration (MIC) by micro – dilution method

Minimum inhibitory concentration (MIC) of each plant extracts and antibiotics alone was determined by the micro-dilution method according to the NCCLS (18). Serial double dilutions of the tested plant extracts, as well as the antibiotics, were prepared in Mueller-Hinton broth and transferred into a 96-well microtiter plate over the final concentration range of  $0.125-128.0\ \mu\text{l/ml}$  and  $0.78-200\ \text{ml/ml}$  for antibiotics and plant extracts respectively. Ten  $\mu\text{l}$  of working inoculum suspension was added to the wells. The final volume was 100  $\mu\text{l}$  and the final bacterial concentration was  $5 \times 10^5$  CFU/ml in each well. Two controls were included, a medium with no inoculum for control of sterility, a medium with no plant extracts or antibiotics for control of inoculum viability. The plates were then incubated for 24 hr at  $37^{\circ}\text{C}$ . After incubation, ten  $\mu\text{l}$  of an aqueous 0.5% triphenyl tetrazoliumchloride (TTC) solution (Sigma-Aldrich) was added to each well and further incubated for 1 hr. The plates were then examined to determine a color change. Viable microorganisms interact with the TTC solution to cause a color change from no color to red. MIC was defined as the lowest concentration showing no color change which exhibited complete inhibition of growth (19). The tests were performed in triplicate.

### Evaluation of synergistic effect

The checkerboard broth microdilution method was used for the determination of synergy between the antibiotics and plant extracts. Two fold serial dilutions of the antibiotic and two fold serial dilutions of the plant extracts were prepared for every combination tested and 50  $\mu\text{l}$  aliquots of each component was placed into the wells of the sterile 96-well microtiter plate. The using inoculum concentration was as in MIC determination method. After incubation of microtiter plates at  $37^{\circ}\text{C}$  for 24 hr, 10  $\mu\text{l}$  of an aqueous 0.5% TTC solution was added to each well and further incubated for 1 hr. MIC was defined as the lowest concentration showing no color change which exhibited complete inhibition of growth.

The checkerboard method is often combined with calculation of fractional inhibitory concentration (FIC) index (FICI). The FIC was derived from the lowest concentration of antibiotic and plant extracts combination showing no color change of TTC. FIC value for each agent was calculated using the formula:

$$\text{FICI} = \Sigma \text{FIC} = \text{FIC (antibiotic)} + \text{FIC (plant extract)}$$

**Table 1.** Antibiotics and plant extract MICs

Bacterial strains	MIC of antibiotics µg/ml								MIC of <i>T. spicata</i> extract mg/ml		
	AM		CEX		AMK		CIP		AQ	ET	PE
MDR Sa1	12	R	32	R	16	I	32	R	50	12.5	12.5
MDR Sa2	64	R	32	R	32	R	8	R	50	12.5	12.5
MDR Sa3	32	R	16	I	16	I	16	R	25	12.5	6.25
MDR Sa4	64	R	16	I	16	I	32	R	25	6.25	6.25
Sen. Sa5	16	R	4	S	4	S	0.5	S	12.5	6.25	6.25
Sa ATCC 25923	1	R	4	S	2	S	0.5	S	25	6.25	6.25
MDR Kp1	32	R	128	R	64	R	32	R	100	100	12.5
MDR Kp2	16	I	64	R	8	I	16	R	50	50	12.5
MDR Kp3	32	R	128	R	32	R	64	R	100	50	12.5
MDR Kp4	8	S	64	R	16	I	32	R	100	50	12.5
Sen. Kp5	4	S	2	S	4	S	0.5	S	25	12.5	3.125
Kp ATCC 700603	4	S	4	S	2	S	0.5	S	50	50	25

Sa: *Staphylococcus aureus*, Kp: *Klebsiella pneumoniae*, AM: ampicillin, CEX: cefotaxime, AMK: amikacin, CIP: ciprofloxacin AQ: aqueous, ET: ethanol, PE: petroleum ether

Where: FIC (antibiotic)= MIC of antibiotic in combination/ MIC of antibiotic alone

FIC (extract) = MIC of extract in combination/ MIC of extract alone

The interactions were classified as being synergistic for  $\Sigma$ FIC values of  $\leq 0.5$ , additive ( $\geq 0.5-1.0$ ), indifferent ( $\geq 1.0-\leq 4.0$ ) or antagonistic ( $\Sigma$ FIC  $> 4.0$ ) (20).

## Results

### Antibacterial activity assay

This study investigated the antimicrobial activities of the phytochemicals extracted from *T. spicata* alone and in combination with four antibiotics against 6 strains of *S. aureus* and 6 strains *K. pneumoniae*. Antibiotic resistance profile of tested strains that presented in Table 1 revealed that four clinical strains of *S. aureus* (Sa1-4) and four clinical strains of *K. pneumoniae* (Kp 1-4) were multidrug-resistant to tested antibiotics Table 1.

The MICs results of *T. spicata* water, ethanol, petroleum ether extracts showed that phytochemicals inhibited the growth of all the bacterial strains under investigation at different extent. The extracts showed antibacterial properties that depend both on the strain and type of extract and solvent, (1% DMSO) did not inhibit the growth of tested strains. The most effective against MDR strains was petroleum ether extract as MICs ranged from 6.25 to 12.5 mg/ml for *S. aureus* strains and 12.5 mg/ml for *K. pneumoniae* strains.

The aqueous extract was the least effective and MICs ranged from 25 to 50 mg/ml and 50 to 100 mg/ml for *S. aureus* and *K. pneumoniae* MDR strains respectively. The MICs of *T. spicata* extracts for ATCC and sensitive strains of *S. aureus* resemble that for MDR strains and ranged from 6.25 to 25 mg/ml, whereas the MICs of plant extracts for ATCC and sensitive strains of *K. pneumoniae* were slightly less than that for MDR strains and ranged from 3.12 to 100 mg/ml.

### Evaluation of synergistic effect of antibiotics/extract

The combination of ampicillin, cefotaxime and amikacin plus plant extracts have synergistic effects on all studied strains of *S. aureus*. Whereas, the joint activity of ciprofloxacin with plant extract showed mostly additive and indifferent effect. The best synergistic capacity against *S. aureus* strains appeared with cefotaxime followed by ampicillin. The interaction between plant extracts was better for resistant strains than sensitive strains as the FIC indices of cefotaxime /antibiotic combination for example were ranged for resistant strains (0.02 to 0.26) comparing with (0.19 to 0.38) for sensitive strains.

The results of a combination of plant extracts against *K. pneumoniae* strains were less effective than against *S. aureus* strains as the most combinations exhibited indifferent effect. The best synergistic capacity against *K. pneumoniae* strains appeared with ampicillin and the FIC indices were ranged from 0.25 to 0.5 for resistant strains.

However, with sensitive strains ampicillin-plant extracts mixture showed additive reaction Table 3. Cefotaxime showed synergism with all *T. spicata* extracts for MDR Kp1 and Kp2 and additive effect for the other strains. Ethanol extract in contrast to other extracts was found to be synergistically effective in combination with amikacin against MDR Kp1 and ATCC KP. Ciprofloxacin has additive and indifference effect with resistant and sensitive *K. pneumoniae* strains when combined with all *T. spicata* extracts.

Among plant extracts, ethanol extract demonstrated best results with different tested antibiotic. Ethanol extract exhibited synergy with ampicillin for ten of twelve strains. cefotaxime/ ethanol extract and amikacin/ ethanol extract showed synergism with nine and eight of tested strains respectively. The mean FIC indices for combinations of ampicillin with aqueous or petroleum ether extracts were from 0.12 to 0.75 and 0.16 to 0.75 respectively.

## Discussion

*S. aureus* is recognized as one of the major causes of infections in humans occurring in both the community and the hospital. Multidrug-resistant staphylococci have become a major nosocomial pathogen; infections are very difficult to cure because strains are resistance against almost all clinically available antibiotics (21).

In addition, *K. pneumoniae* is a frequent cause of nosocomial infections and has also emerged as an agent of severe community-acquired infections, including pyogenic liver abscess, pneumonia, and meningitis (22). Antimicrobial drugs effective for treatment of infection with MDR bacteria are limited. Thus, it is valuable to find compounds that potentiate antimicrobial activity of antibiotics on these bacteria. The ability of plant extracts to act synergistically with antibiotics is considered a new approach that helps in solving the problem of bacterial resistance (5, 10, 23).

**Table 2.** FICs of *S. aureus* strains for the antibiotics/*Thymbra spicata* extract combinations

<i>S. aureus</i> strains	FICs	AM			CTX			AMK			CIP		
		(AQ)	(ET)	(PE)	(AQ)	(ET)	(PE)	(AQ)	(ET)	(PE)	(AQ)	(ET)	(PE)
MDR Sa1	FIC (Ab)	0.06	0.01	0.02	0.002	0.01	0.06	0.03	0.06	0.03	0.5	0.25	0.25
	FIC (Ts)	0.06	0.06	1.25	0.02	0.06	0.06	0.06	1.13	0.06	0.5	0.5	0.5
	ΣFIC	<b>0.12</b>	<b>0.07</b>	<b>0.27</b>	<b>0.02</b>	<b>0.07</b>	<b>0.12</b>	<b>0.09</b>	<b>0.18</b>	<b>0.09</b>	1	0.75	0.75
MDR Sa2	FIC (Ab)	0.13	0.03	0.03	0.06	0.06	0.06	0.06	0.03	0.06	1	0.13	0.13
	FIC (Ts)	0.13	0.13	0.13	0.06	0.06	0.06	0.13	0.13	0.13	0.25	0.5	0.5
	ΣFIC	<b>0.26</b>	<b>0.16</b>	<b>0.16</b>	<b>0.12</b>	<b>0.12</b>	<b>0.12</b>	<b>0.19</b>	<b>0.16</b>	<b>0.19</b>	1.25	0.63	0.63
MDR Sa3	FIC (Ab)	0.25	0.13	0.13	0.13	0.06	0.06	0.13	0.06	0.25	0.25	0.25	0.25
	FIC (Ts)	0.13	0.13	0.13	0.13	0.06	0.13	0.06	0.13	0.06	0.75	0.5	1
	ΣFIC	<b>0.38</b>	<b>0.26</b>	<b>0.26</b>	<b>0.26</b>	<b>0.12</b>	<b>0.19</b>	<b>0.19</b>	<b>0.19</b>	<b>0.31</b>	0.75	0.75	1.25
MDR Sa4	FIC (Ab)	0.06	0.13	0.25	0.13	0.13	0.13	0.13	0.13	0.13	0.25	0.13	0.13
	FIC (Ts)	0.06	0.25	0.13	0.13	0.13	0.13	0.13	0.25	0.25	0.5	0.5	0.5
	ΣFIC	<b>0.12</b>	<b>0.38</b>	<b>0.38</b>	<b>0.26</b>	<b>0.26</b>	<b>0.26</b>	<b>0.26</b>	<b>0.38</b>	<b>0.38</b>	0.75	0.63	0.63
Sen. Sa5	FIC (Ab)	0.13	0.06	0.13	0.13	0.03	0.06	0.25	0.13	0.13	1	0.5	0.25
	FIC (Ts)	0.5	0.25	0.13	0.25	0.25	0.13	0.13	0.13	1.13	0.12	0.25	0.5
	ΣFIC	0.63	<b>0.31</b>	<b>0.26</b>	<b>0.38</b>	<b>0.28</b>	<b>0.19</b>	<b>0.38</b>	<b>0.26</b>	<b>0.26</b>	1.12	0.75	0.75
ATCC 25923	FIC (Ab)	0.25	0.13	0.25	0.13	0.06	0.13	0.25	0.13	0.13	1	0.5	1
	FIC (Ts)	0.25	0.13	0.5	0.13	0.13	0.13	0.25	0.25	0.25	0.25	0.5	0.5
	ΣFIC	<b>0.5</b>	<b>0.26</b>	0.75	<b>0.26</b>	<b>0.19</b>	<b>0.26</b>	<b>0.5</b>	<b>0.38</b>	<b>0.38</b>	1.25	1	1.5

Sa: *Staphylococcus aureus*, Kp: *Klebsiella pneumoniae*, AM: ampicillin, CEX: cefotaxime, AMK: amikacin, CIP: ciprofloxacin  
AQ: aqueous, ET: Ethanol, PE: petroleum ether

**Table 3.** FICs of *K. pneumoniae* strains for the antibiotics/*Thymbra spicata* extract combinations

<i>K.pneumoniae</i> strains	FICs	AM			CTX			AMK			CIP		
		(AQ)	(ET)	(PE)	(AQ)	(ET)	(PE)	(AQ)	(ET)	(PE)	(AQ)	(ET)	(PE)
MDR Kp1	FIC (Ab)	0.25	0.13	0.13	0.25	0.13	0.13	0.25	0.25	0.13	0.5	0.5	0.25
	FIC (Ts)	0.25	0.13	0.25	0.25	0.13	0.25	0.5	0.25	0.5	0.5	0.5	1
	ΣFIC	<b>0.5</b>	<b>0.26</b>	<b>0.38</b>	<b>0.5</b>	<b>0.26</b>	<b>0.38</b>	0.75	<b>0.5</b>	0.63	1	1	1.25
MDR Kp2	FIC (Ab)	0.25	0.13	0.13	0.25	0.13	0.13	0.5	0.25	0.25	1	0.25	0.25
	FIC (Ts)	0.25	0.13	0.13	0.25	0.25	0.25	0.5	0.5	1	1	0.5	0.5
	ΣFIC	<b>0.5</b>	<b>0.26</b>	<b>0.26</b>	<b>0.5</b>	<b>0.38</b>	<b>0.38</b>	1	0.75	1.25	1.5	0.75	0.75
MDR Kp3	FIC (Ab)	0.5	0.25	0.25	0.25	0.13	0.13	0.25	0.25	0.13	0.5	0.25	0.13
	FIC (Ts)	0.25	0.25	0.25	0.5	0.5	0.5	1	1	1	1	0.5	1
	ΣFIC	0.75	<b>0.5</b>	<b>0.5</b>	0.75	0.63	0.63	1.25	1.25	1.13	1.5	0.75	1.13
MDR Kp4	FIC (Ab)	0.25	0.13	0.13	0.25	0.13	0.13	0.5	0.5	0.25	0.5	0.25	0.25
	FIC (Ts)	0.25	0.13	0.5	0.5	0.25	0.5	0.5	1	0.5	1	1	1
	ΣFIC	<b>0.5</b>	<b>0.26</b>	0.63	0.75	<b>0.38</b>	0.63	1	1.5	0.75	1.5	1.25	1.25
Sen. Kp5	FIC (Ab)	0.5	0.5	0.5	0.5	0.25	0.25	0.5	0.5	0.25	1	1	1
	FIC (Ts)	0.5	0.5	1	0.5	0.5	1	0.5	0.5	1	1	1	1
	ΣFIC	1	1	1.5	1	0.75	1.25	1	1	1.25	2	2	2
ATCC 700603	FIC (Ab)	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25	0.25	1	1	1
	FIC (Ts)	0.5	0.5	0.5	1	0.5	0.5	1	0.25	1	1	1	1
	ΣFIC	0.75	0.75	0.75	1.25	0.75	0.75	1.5	<b>0.5</b>	1.25	2	2	2

Sa: *Staphylococcus aureus*, Kp: *Klebsiella pneumoniae*, AM: ampicillin, CEX: cefotaxime, AMK: amikacin, CIP: ciprofloxacin, AQ: aqueous, ET: ethanol, PE: petroleum ether

In current study, the MIC of plant extracts against MDR *S. aureus* and *K. pneumoniae* strains ranged from 6.25 to 50 and 12.5 to 100 mg/ml respectively, which reflects a moderate antibacterial effect against studied strains. These findings are consistent with those obtained in some previous studies to a certain degree considering the antibacterial effect of the essential oils of *T. spicata* (24-26). Nevertheless, the antibacterial effect of the water, ethanol and petroleum ether extracts of *T. spicata* was seldom evaluated against MDR *S. aureus* and *K. pneumoniae* clinical isolates and most previous studies on the antibacterial activity of *T. spicata* were on its essential oils. The MICs values of plant extracts against sensitive and resistant strains were convergent. It was reported that bacterial strain in the same species that are sensitive to antibiotics, exhibit higher sensitivity to plant extracts than resistant (27, 28). This may be due to the difference in modes of action of various compounds present in the extracts, to which the organism was never exposed before and hence never had a chance to develop resistance. The antimicrobial properties of alcoholic and petroleum ether extract were superior compared to aqueous extract for those

selected bacteria. This finding was in accordance with previous research which had reported that the plant extracts using organic solvent exhibited more antibacterial activity compared to the extract using aqueous as a solvent (29).

The antibacterial effect of *T. spicata* extracts was more evident against *S. aureus* strains than *K. pneumoniae* strains. Similar to current study, Omar et al (2013) reported that *S. aureus* and *K. pneumoniae* were sensitive to aqueous extract of *T. spicata* (MIC= 50 mg/ml) but *K. pneumoniae* was resistant to ethanolic extract (30). In addition, Bakhtiyari et al (2014) found Gram-negative bacteria to be less susceptible to hydroalcoholic extract and essential oil of *T. spicata* than Gram-positive bacteria (31). This difference could be explained by the presence of an outer membrane in Gram negative bacteria, the lipopolysaccharide layer that hindered the access of most compounds to the bacterial cell (32).

Although the antibacterial activity *T. spicata* is reported (24-26) the amount of data published about its effectiveness against multidrug-resistant pathogens is very scanty. Consequently, the present study was focused on the antibacterial as well as synergistic activity of plant extracts with antibiotics.



The results revealed the synergistic and additive interactions between *T. spicata* phytochemicals and antibiotics, which are cell wall inhibitors (ampicillin, cefotaxime), protein synthesis inhibitor (amikacin), and DNA synthesis inhibitor (ciprofloxacin). Beta-lactam antibiotics were one of the antimicrobial groups that most commonly associated with a positive interaction potential, which was also approved in this current study (33-35). The major mechanism of  $\beta$ -lactam resistance has involved  $\beta$ -lactamases and altered penicillin binding proteins (PBPs) (36, 37).

The exact mechanism for the reduction of  $\beta$ -lactam resistance by the natural antimicrobials is unknown but it maybe due to some structural change in the resistant bacteria, inhibition of the activity of penicillinase (37) or inhibition of the activity of altered PBP production (38). The resistance to aminoglycoside is caused by three mechanisms: reduced uptake or decrease permeability, alteration at ribosomal binding sites, or production of modifying enzymes (39). The combination of amikacin with *T. spicata* extracts resulted in synergistic activity when tested against all *S. aureus* strains. However, ethanolic extract showed synergism with amikacin against only two strains (ATCC and MDR Kp1) of *K. pneumoniae* where the activity of amikacin increase four fold. The difference between two species may explained by different type of resistance mechanisms.

As in the present study, Atteiaa and Husseinb (2014) noticed better synergism between ethanolic extract from *Syzygium aromaticum* (clove) and *Allium sativum* (garlic) with different used antibiotics comparing with water extract against *S. aureus* and *K. pneumoniae* isolates (40). However, the combination between antibiotics and extracts was tested by disk-diffusion method and synergistic effect was observed on the basis of enlargement of inhibition zone.

Nucleic acid synthesis inhibitor commonly showed indifference effect with all extracts and against all studied strains. Ciprofloxacin and ofloxacin were reported to have a good synergistic activity with different phytochemical compound in previous studies which contraindicated with our results (35, 41). However, different other study reported none or weak synergism activity between nucleic acid synthesis inhibitor and plant extracts (33, 41).

The MICs of antibiotics against studied MDR strains demonstrated high level of resistance to common antibiotics. All the extracts of the studied plant showed an increase in the antimicrobial activity of certain drugs that can be used against *S. aureus* or *K. pneumoniae*. The average increase in activity of antibiotic against resistant *S. aureus*

ranged from 8-128 fold for cefotaxime and from 4-16 fold for amikacin.

The average increase in activity of antibiotic against resistant *K. pneumoniae* ranged from 4-8 fold for ampicillin, cefotaxime and amikacin. The synergistic effect between antimicrobial agent and plant extracts was occurred in both sensitive and resistant strains, but the MIC was decreased more in resistant than sensitive strains. The average increase in activity of antibiotic against sensitive strains ranged from 2-16 fold for *S. aureus* and 2 fold for *K. pneumoniae*. It was reported that combination of two agents exhibit significant synergism only if the test organism is resistant to at least one of the agents (43, 44). The current study findings are consistent with previous reports which showed that some plant extracts can increase the activity of antimicrobial drugs *in vitro* against bacteria (24, 33, 45-48). The synergistic interaction observed between *T. spicata* extracts and the evaluated antibiotics can be translated into useful clinical applications in *S. aureus* and *K. pneumoniae* infections. Essential oil is one of the main compounds of this herb which exists in 1-2%. It's characterized by a high content of carvacrol,  $\gamma$ -terpinene and *p*-cymene (14, 15, 24). The synergistic activity of *T. spicata* extracts may attribute to ability of active component like carvacrol to disturb the cell wall and depolarize the cytoplasmic membrane and then facilitate the influx of antibiotics inside bacterial cell (48, 49).

The combinations of antibiotics with plant extracts could be a significant basis for development of new approach in resistance modifying agents because the use of extracts shows a low risk of increasing bacterial resistance to their action. Actually, the extracts contain mixtures of different bioactive compounds, which make microbial adaptability very difficult comparing to single-constituent antibiotics (12).

## Conclusion

*T. spicata* extracts are found to have the capability of increasing the susceptibility of the studied strains to various antibiotics. The present study clearly suggests the possibility of use of the above shown synergistic drug-herb combinations for combating infections caused by MDR strains.

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## References

1. Boucher HW, Talbot GH, Bradley JE, Edwards Jr, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no escape! An update from the infectious diseases society of America. Clin Infect Dis 2009; 48:1-12.

2. Harbarth S, Samore MH. Antimicrobial resistance determinants and future control. *Emerg Infect Dis* 2005; 11:794-801.
3. Spellberg B, Bartlett JG, Gilbert DN. The future of antibiotics and resistance. *N Engl J Med* 2013; 368:299-302.
4. Torella JP, Chait R, Kishony R. Optimal drug synergy in antimicrobial treatments. *PLoS Comput Biol* 2010; 6:1-8.
5. Sibanda T, Okoh AI. The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. *Afr J Biotechnol* 2007; 6:2886-2896.
6. Adwan G, Abu-Shanab B, Adwan K. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug-resistant *Pseudomonas aeruginosa* strains. *Asian Pac J Trop Biomed* 2010; 1:266-269.
7. Darwish RM, Aburjai TA. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escherichia coli*. *BMC Complement Altern Med* 2010; 10:9-16.
8. Hübsch Z, van Zyl RL, Cock IE, van Vuuren SF. Interactive conventional antimicrobial and toxicity profiles of antimicrobials with Southern African medicinal plants. *S Afr J Bot* 2014; 93:185-197.
9. Bassolé IH, Juliani HR. Essential oils in combination and their antimicrobial properties. *Molecules* 2012; 17:3989-4006.
10. Aiyegoro OA, Okoh AI. Use of bioactive plant products in combination with standard antibiotics: Implications in antimicrobial chemotherapy. *J Med Plant Res* 2009; 3:1147-1152.
11. Gibbons S. Anti-staphylococcal. *Nat Prod Rep* 2004; 21:263-277.
12. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine* 2008; 15:639-652.
13. Mouterde P. Nouvelle flore du Liban et de la Syrie. Tome III, Dar El Mashreq, Liban: 1983.
14. Barakata A, Wakimb LH, Apostolidesb NA, Sroua G, El Beyrouthyb M. Variation in the essential oils of *Thymbra spicata* L. growing wild in Lebanon, according to the date of harvest. *J Essent Oil Res* 2013; 25:506-511.
15. Marković T, Chatzopoulou P, Šiljegović J, Nikolic M, Glamo J, Ćirić A, et al. Chemical analysis and antimicrobial activities of the essential oils of *Satureja thymbra* L. and *Thymbra spicata* L. and their main components. *Arch Biol Sci* 2011; 63:457-464.
16. Celiktas OY, Kocabas EEH, Bedir E, Sukan FV, Ozek T, Baser KHC. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chem* 2007; 100:553-559.
17. Balouiri M, Sadiki M, Ouedehiri W, Farah A, Abed S, Koraichi SI. Antibacterial activity of extracts from *Salvia officinalis* and *Rosmarinus officinalis* obtained by sonication and maceration methods. *Int J Pharm Pharm Sci* 2014; 6:167-170.
18. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Pennsylvania: NCCLS 2013; M100-S23.
19. Klančnik A, Piskernik S, Jeršek B, Možina SS. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *J Microbiol Methods* 2010; 81:121-126.
20. Vuuren S, Viljoen A. Plant-based antimicrobial studies—methods and approaches to study the interaction between natural products. *Planta Med* 2011; 77:1168-1182.
21. Styers CD, Sheehan DJ, Hogan P, Sahn DF. Laboratory-based surveillance of current antimicrobial resistance patterns and trends among *Staphylococcus aureus*: 2005 status in the United States. *Ann Clin Microbiol Antimicrob* 2006; 5:1-9.
22. Porsche R, Ullmann U. *Klebsiella spp.* as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* 1998; 11:589-603.
23. Khameneh B, Diab R, Ghazvini K, Fazly Bazzaz BS. Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. *Microb Pathog* 2016; 95:1-11.
24. Kilic TZ. Analysis of essential oil composition of *Thymbra spicata* var. *spicata*: antifungal, antibacterial and antimycobacterial activities. *Naturforsch* 2006; 61:324-328.
25. Akin M, Oguz D, Saracoglu TH. Antibacterial effect of essential oil from *Thymbra spicata* var. *spicata* L. and *Teucrium polium* (Stapf Brig). *Int J Pharm Appl Sci* 2010; 1:55-58.
26. Markovic T, Chatopoulou P, Siljegovic J. Chemical analysis and antimicrobial activities of the essential oils of *Satureja thymbra* L. and *Thymbra spicata* L. and their main components. *Arch Biol Sci* 2011; 63:457-464.
27. Jahan F, Lawrence R, Kumar V, Junaid M. Evaluation of antimicrobial activity of plant extracts on antibiotic-susceptible and resistant *Staphylococcus aureus* strains. *J Chem Pharm Res* 3:777-789.
28. Nascimento GGF, Locatelli, J Friestas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz J Microbiol* 2000; 31:345-353.
29. Parekh J, Jadeja D, Hanlin RL. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turk J Biol* 2005; 29:203-210.
30. Omar G, Abdallah LA, Ismail S, Almasri MY. Screening of selected medicinal wild plant extracts antibacterial effect as natural alternatives. *Inter J Indig Med Plants* 2013; 46:1299-1304.
31. Bakhtiyari S, Sabzali S, Rostamzad A, Basati G. Investigating antimicrobial activity of hydroalcoholic extract and essential oil of *Thymbra spicata* against some pathogenic bacteria. *J Basic Res Med Sci* 2014; 1-7.
32. Ilić BS, Kocić BD, Ćirić VM, Ćvetković OG, Miladinović DL. An *in vitro* synergistic interaction of combinations of *thymus glabrescens* essential oil and its main constituents with chloramphenicol. *ScientificWorldJournal* 2014; 1:1-12.
33. Betoni JE, Mantovani RP, Barbosa LN, Di Stasi LC, Fernandes JA. Synergism between plant extract and

antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem Inst Oswaldo Cruz 2006; 10:387-390.

34. Abd El-Kalek HH, Mohamed EA. Synergistic effect of certain medicinal plants and amoxicillin against some clinical isolates of methicillin - resistant *Staphylococcus aureus* (MRSA). Int J Pharm Appl 2012; 3:387-398.

35. Hübsch Z, van Zyl RL, Cock IE, van Vuuren SF. Interactive antimicrobial and toxicity profiles of conventional antimicrobials with Southern African medicinal plants. S Afr J Bot 2014; 93:185-197.

36. Zhao WH, Hu ZQ, Okubo S, Hara Y, Shimamura T. Mechanism of synergy between Epigallochatechin gallate and  $\beta$ -Lactams against methicillin resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother 2012; 45:1737-1742.

37. Esimone CO, Iroha IR, Ibezim EC, Okeh CO, Okpana EM. *In vitro* evaluation of the interaction between tea extracts and penicillin G against *Staphylococcus aureus*. Afr J Biotechnol 2006; 5:1082-1086.

38. Georgopapadakou NH. Penicillin-binding proteins and bacterial resistance to  $\beta$ -Lactams. Antimicrob Agents Chemother 1993; 37:2045-2053.

39. Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. Aminoglycosides; Activity and resistance. Antimicrob Agents Chemother 1999; 43:727-737.

40. Atteiaa HG, Husseinb EM. *In vitro* antibacterial and synergistic effects of some plant extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*. J Antimicrob 2014; 129:338-346.

41. Chatterjee SK, Bhattacharjee I, Chandra G. *In vitro* synergistic effect of doxycycline and ofloxacin in

combination with ethanolic leaf extract of *Vangueria spinosa* against four pathogenic bacteria. Indian J Med Res 2009; 130:475-478.

42. Ghaly MF, Shalaby MA, Shas SM, Shehata MN, Ayad AA. Synergistic effect of antibiotics and plant extract to control clinical bacterial isolates implicated in urinary tract infections. J Appl Sci Res 2009; 5:1298-1306.

43. Chovanová R, Mikulášová M, Vaverková Š. *In vitro* antibacterial and antibiotic resistance modifying effect of bioactive plant extracts on methicillin-resistant *Staphylococcus epidermidis*. Int J Microbiol 2013; 2013:760969.

44. Horiuchi K, Shiota S, Kuroda T, Hatano T, Yoshida T, Tsuchiya T. Potentiation of antimicrobial activity of aminoglycosides by carnosol from *Salvia officinalis*. Biol Pharm Bull 2007; 30:287-290.

45. Adwan G, Mhanna M. Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. Middle East J Sci Res 2008; 3: 134-139.

46. Ahmed Z, Khan SS, Khan M, Tanveer A, Lone ZA. Synergistic effect of *Salvadora persica* extracts, tetracycline and penicillin against *Staphylococcus aureus*. Afr J Basic Appl Sci 2010; 2:25-29.

47. Cristiani M, D'Arrigo M, Mandalari G, Castelli F, Sarpietro MG, Micieli D. Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. J Agric Food Chem 2007; 55:6300-6308.

48. Xu J, Zhou F, Ji BP, Pei RS, Xu N. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. Lett Appl Microbiol 2008; 47:174-179.