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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2015.09.024

Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants

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ARTICLE INFO

Article history:
Received 9 Mar 2015
Received in revised form 17 Apr, 2nd revised form 26 Aug 2015
Accepted 19 Sep 2015
Available online 14 Nov 2015

Keywords:
Essential oils
Antibacterial
Synergies
Additive
Antagonistic

ABSTRACT

Objective: To demonstrate the *in vitro* antibacterial properties of five essential oils against ten bacterial strains and study the synergistic effect of the combination of essential oils with standard antibiotics.

Methods: *Origanum compactum*, *Chrysanthemum coronarium*, *Thymus willdenowii* Boiss, *Melissa officinalis* and *Origanum majorana* L. were used alone and combined used with standard antibiotics to evaluate their antimicrobial activities. The disk diffusion method was employed.

Results: The results showed that the combined application of the essential oils of the plants with antibiotics led to a synergistic effect in some cases, but antagonistic effect was also observed in some bacteria.

Conclusions: This study shows that the combination of essential oils of the five plants with antibiotics may be useful in the fight against emerging microbial drug resistance.

1. Introduction

Antibiotic resistance is a phenomenon as old as the advent of antibiotics. Antibiotics are from natural substances produced by fungi but also by certain bacteria to “defend” against other bacteria. The bacteria are not suicidal; the first who learned to synthesize antibiotics developed at the same time the means to protect themselves [1]. The development and spread of resistance to currently available antibiotics is a global concern [2]. With the increase in bacterial resistance to antibiotics, antimicrobials plant products have gained attention in the scientific research. The use of natural antimicrobial compounds is important not only in food preservation, but also in the control of human diseases and plant microbial origin [3]. The use of natural products with therapeutic properties, whether mineral, vegetable and animal, for a long time were the main sources of important therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicines [4]. Medicinal plants are considered an important source of new chemical substances with potential therapeutic effects [5]. They contain a wide range of substances that can be used to treat chronic diseases, and infectious diseases. Essential oils are a very interesting group of secondary metabolites that are potentially useful sources of antimicrobial compounds. Many studies have been published on the antimicrobial activity of essential oils [6–9].

According to Enrico *et al.* [10], the essential oils, unlike antibiotics, are composed of many molecules so that bacteria cannot resist in mutant. Preventively and curatively, they are especially known for their potent antibacterial, antiviral, anti inflammatory, anti fungal, anti parasitic, antipyretic, expectorant, and mucolytic effects. The combination of essential oils with antibiotics therapeutic approach may lead to new ways to treat infectious diseases. Many researchers have studied experimentally the synergistic effect resulting from the combination of antibiotics with different plant extracts [11–14]. Indeed this combination therefore allowed reducing bacterial resistance to drugs [15]. This work was carried out in order to demonstrate the *in vitro* antibacterial properties of five essential oils against ten bacterial strains by disc diffusion method and study the synergistic effect of the combination of essential oils with standard antibiotics.

2. Materials and methods

2.1. Plant materials

Samples of *Origanum compactum* (*O. compactum*), *Chrysanthemum coronarium* (*C. coronarium*) and *Thymus*

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Peer review under responsibility of Hainan Medical University.

willdenowii Boiss (*T. willdenowii*) were harvested in Khénifra while *Melissa officinalis* (*M. officinalis*) and *Origanum majorana* L. (*O. majorana*) were harvested in the Marrakech Region. The sample collection was conducted in the months of May to June, 2014. The samples were dried in the shade for 10 days before the steam distillation.

2.2. Hydrodistillation

The extraction of the essential oils was carried out by hydrodistillation in a Clevenger-type apparatus [16]. The essential oils were stored at 4 °C in the dark and in the presence of anhydrous sodium sulfate.

2.3. Microorganisms

The antibacterial activity was evaluated using Gram positive bacteria: *Staphylococcus aureus* (*S. aureus*), and Gram negative bacteria: *Escherichia coli* (*E. coli*), *E. coli* (ATCC 25921), *Klebsiella pneumoniae* (*K. pneumoniae*), *Proteus mirabilis* (*P. mirabilis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *P. aeruginosa* (ATCC 27853), *Pseudomonas putida* (*P. putida*), *Salmonella enteritidis* (*S. enteritidis*) and *Enterobacter aerogenes* (*E. aerogenes*). All bacterial strains were provided from the microbiology laboratory of the hospital Mehemmed VI. Bacterial strains were maintained by subculture on nutrient agar favorable to their growth for 24 h in the dark at 37 °C.

2.4. Antibiotics

The antibiotics–standard gentamicin (10 µg), tobramycin (30 µg), imipenem (10 µg) and ticarcillin (75 µg) were used.

2.5. Antimicrobial activity

The antimicrobial activity of the extracts was determined by the disk diffusion method which is based on the spread of antimicrobial compound in solid medium [17]. The Mueller–Hinton agar was poured in sterile petri dishes (90 mm diameter). The paper discs (6 mm diameter) that were impregnated with 2 µL of each pure essential oil and antibiotic and tested standard discs were placed on the inoculated agar surface. Petri dishes were allowed to stand for 30 min at room temperature before incubation at 37 °C for 24 h. The effect of essential oils was reflected by the appearance around disc with a transparent circular zone corresponding to the absence of growth. The diameter of inhibition zone was measured in mm. The larger the diameter of the area the more susceptible the strain [18]. To evaluate the synergistic effect of the combination of the essential oils and antibiotics which are in the form of ready to use discs, 2 µL of each essential oil was saturated to the antibiotic disc to determine the zones of inhibition [19]. The obtained results were compared with those of the antibiotics tested on the same strains alone and by the same method.

2.6. Statistical analysis

All experiments were repeated three times. Results were presented as mean ± SEM.

3. Results

3.1. T. willdenowii Boiss

T. willdenowii Boiss essential oil showed significant antibacterial characters against the tested microorganisms with exception of *P. putida*, *P. aeruginosa* and *P. aeruginosa* ATCC 27853 (Table 1).

According to the obtained results, the combination of essential oil of *T. willdenowii* Boiss with tobramycin antibiotics showed an antagonistic effect against six tested bacteria (Table 1). A synergistic effect was observed in *K. pneumoniae*, *P. aeruginosa*, *P. aeruginosa* ATCC 27853, *P. putida* and *E. aerogenes*. The application of ticarcillin with the essential oil of *T. willdenowii* Boiss led to a synergistic effect on *E. coli* (ATCC 25921), *E. coli*, *P. putida*, *S. enteritidis*, *E. aerogenes* and *P. aeruginosa*. An antagonistic effect was observed in other bacteria. Combination of the essential oil of *T. willdenowii* Boiss with imipenem had an antagonistic effect against *K. pneumoniae*, *E. aerogenes*, *P. mirabilis*, and a synergistic effect against *E. coli*, *E. coli* (ATCC 25921), *P. putida*, *P. aeruginosa*, *S. enteritidis* and *P. aeruginosa* ATCC 27853. A synergistic effect was observed in *P. putida*, *S. enteritidis*, an undifferentiated effect in *E. coli* and an antagonistic effect were observed on the other tested bacteria when there was a combination of the essential oil of *T. willdenowii* Boiss and gentamisine.

Table 1

The antimicrobial activities (zones of inhibition) of essential oil of *T. willdenowii* and its synergistic effect with antibiotics. mm.

Microorganisms	Essential oil	Standard antibiotic discs				Essential oil and standard antibiotic discs			
		TOB	TIC	IPM	G	TOB	TIC	IPM	G
E. coli	12.33 ± 0.57	20.33 ± 1.52	–	24.33 ± 0.57	20.33 ± 0.57	23.00 ± 1.00 A	16.00 ± 1.00 S	37.33 ± 0.57 S	20.33 ± 0.57 I
E. coli (ATCC 25921)	12.33 ± 0.57	22.33 ± 0.57	–	20.00 ± 0.00	22.66 ± 0.15	27.33 ± 1.52 A	14.33 ± 0.57 S	34.33 ± 1.52 S	24.00 ± 0.15 A
K. pneumoniae	20.00 ± 0.00	10.00 ± 0.00	20.66 ± 1.15	24.33 ± 0.57	22.00 ± 0.00	32.00 ± 0.00 S	34.33 ± 1.52 A	26.00 ± 0.57 A	32.00 ± 0.00 A
E. aerogenes	19.00 ± 0.00	10.00 ± 0.00	7.00 ± 0.00	17.66 ± 0.57	10.33 ± 0.57	30.00 ± 2.00 S	34.66 ± 0.57 S	22.66 ± 0.57 A	24.00 ± 0.57 A
S. aureus	13.00 ± 0.00	21.33 ± 0.57	–	–	22.33 ± 0.57	25.33 ± 0.57 A	–	–	23.00 ± 0.57 A
P. mirabilis	10.33 ± 0.57	9.00 ± 0.00	19.00 ± 0.57	27.00 ± 0.00	8.33 ± 0.57	10.66 ± 0.57 A	17.33 ± 0.57 A	26.00 ± 0.00 A	9.00 ± 0.57 A
P. putida	–	7.33 ± 0.57	14.00 ± 0.00	26.66 ± 0.57	–	20.00 ± 1.73 S	21.33 ± 0.57 S	27.00 ± 0.57 S	12.00 ± 0.00 S
P. aeruginosa	–	21.33 ± 0.57	24.00 ± 0.00	24.00 ± 0.00	20.33 ± 0.57	32.33 ± 0.57 S	17.00 ± 0.00 A	25.00 ± 0.00 S	7.00 ± 0.57 A
P. aeruginosa (ATCC 27853)	–	23.00 ± 1.00	23.66 ± 0.57	30.66 ± 1.15	18.66 ± 0.57	26.00 ± 0.00 S	22.33 ± 1.52 A	32.00 ± 0.00 S	17.00 ± 0.57 A
S. enteritidis	13.00 ± 0.00	21.33 ± 0.57	–	–	12.33 ± 0.57	22.66 ± 0.57 A	18.33 ± 0.57 S	20.00 ± 1.00 S	16.00 ± 0.57 S

Values are represented as mean ± SEM; I: Indifference; S: Synergy; A: Antagonism; TOB: Tobramycin; TIC: Ticarcillin; IPM: Imipenem; G: Gentamicin.

Table 2
The antimicrobial activities (zones of inhibition) of essential oil of *O. compactum* L. and its synergistic effect with antibiotics. mm.

Microorganisms	Essential oil	Standard antibiotic discs				Essential oil and standard antibiotic discs			
		TOB	TIC	IPM	G	TOB	TIC	IPM	G
E. coli	15.33 ± 0.57	20.33 ± 1.52	–	24.33 ± 0.57	20.33 ± 0.57	25.00 ± 1.00 A	16.33 ± 0.57 S	32.33 ± 2.51 A	22.00 ± 0.00 A
E. coli (ATCC 25921)	13.00 ± 0.00	22.33 ± 0.57	–	20.00 ± 0.00	22.66 ± 0.15	25.33 ± 0.57 A	15.00 ± 0.57 S	31.33 ± 1.52 A	23.00 ± 1.00 A
K. pneumonia	25.66 ± 0.57	10.00 ± 0.00	20.66 ± 1.15	24.33 ± 0.57	22.00 ± 0.00	36.00 ± 0.00 S	38.00 ± 1.15 A	40.00 ± 0.57 A	38.00 ± 0.00 A
E. aerogenes	28.00 ± 0.00	10.00 ± 0.00	7.00 ± 0.00	17.66 ± 0.57	10.33 ± 0.57	28.00 ± 1.00 I	26.00 ± 2.00 A	26.00 ± 1.00 A	27.33 ± 0.57 A
S. aureus	18.33 ± 0.57	21.33 ± 0.57	–	–	22.33 ± 0.57	25.00 ± 0.57 A	–	–	26.00 ± 0.57 A
P. mirabilis	–	9.00 ± 0.00	15.00 ± 0.57	27.00 ± 0.00	8.33 ± 0.57	11.00 ± 0.00 S	18.00 ± 0.57 S	24.00 ± 0.00 A	9.00 ± 0.00 S
P. putida	–	7.33 ± 0.57	14.00 ± 0.00	26.66 ± 0.57	–	10.00 ± 0.57 S	18.00 ± 0.00 S	28.33 ± 0.57 S	9.66 ± 0.57 S
P. aeruginosa	–	21.33 ± 0.57	24.00 ± 0.00	24.00 ± 0.00	20.33 ± 0.57	32.66 ± 1.52 S	26.33 ± 0.57 S	23.00 ± 0.00 A	22.00 ± 1.00 S
P. aeruginosa (ATCC 27853)	–	23.00 ± 1.00	23.66 ± 0.57	30.66 ± 1.15	18.66 ± 0.57	27.00 ± 1.00 S	25.00 ± 0.57 S	–	21.00 ± 0.57 S
S. enteritidis	14.00 ± 0.00	21.33 ± 0.57	–	–	12.33 ± 0.57	24.00 ± 0.57 A	15.00 ± 1.00 S	28.33 ± 0.57 S	17.00 ± 0.57 A

Values are represented as mean ± SEM; I: Indifference; S: Synergy; A: Antagonism; TOB: Tobramycin; TIC: Ticarcillin; IPM: Imipenem; G: Gentamicin.

3.2. *O. compactum* L.

The essential oil of *O. compactum* L. showed a significant inhibitory effect against the studied microorganisms except for *P. aeruginosa*, *P. aeruginosa* (ATCC 27853), *P. mirabilis* and *P. putida* (Table 2).

A synergistic effect was observed in *K. pneumoniae*, *P. putida*, *P. aeruginosa* (ATCC 27853), *P. aeruginosa*, *P. mirabilis*, when there was a combination of *O. compactum* L. with tobramycin, while an antagonistic effect was seen on other bacteria. Ticarcillin with *O. compactum* L. had a synergistic effect in most bacteria, antagonistic effect was observed in *K. pneumoniae* and *E. aerogenes*. Combination of the essential oil of *O. compactum* L. with imipenem antibiotic disc led to a synergistic effect against *P. putida* and *S. enteritidis* and an antagonist effect in the rest of bacteria. A synergistic effect was also observed in *P. putida*, *P. mirabilis*, *P. aeruginosa* (ATCC 27853) and *P. aeruginosa* when the combination of essential oils of *O. compactum* L. with gentamicin was applied. The antagonistic effect has been observed in other bacteria.

3.3. *O. majorana* L.

Essential oils of *O. majorana* L. showed an average activity against *E. coli*, *E. coli* (ATCC 25921), *K. pneumoniae*, *E. aerogenes*, *S. auereus* and *S. enteritidis* with zones of inhibitions between (8.00 ± 1.00) mm and (10.00 ± 1.00) mm, while no effect was detected in *P. putida*, *P. mirabilis*, *P. aeruginosa* (ATCC 27853) and *P. aeruginosa* (Table 3).

From Table 3, it can be seen that the combination of the essential oil of *O. majorana* L with antibiotic discs tobramycin led to a synergistic effect against *K. pneumoniae* and *P. putida*. An antagonistic effect was observed in the other tested bacteria. An additive effect was observed in *E. aerogenes*, a synergistic effect in *E. coli* ATCC 25921, *S. enteritidis* and *E. coli*, and an antagonistic effect was seen in the other bacteria when the combination of essential oils of *O. majorana* and ticarcillin was applied. The combination of imipenem with the essential oil of *O. majorana* L. had a synergistic effect in *E. coli* (ATCC 25921), *P. aeruginosa* (ATCC 27853), *P. aeruginosa*, *S. enteritidis* and *P. putida*, while the antagonist effect was in other bacteria tested.

The combination of essential oil with gentamicin led to a synergistic effect against *K. pneumoniae*, an additive effect against *E. aerogenes* and an antagonistic effect against the rest of the bacteria. No inhibitory effect has been recorded on *P. putida, P. mirabilis, P. aeruginosa.*

3.4. *M. officinalis L.*

Essential oils of *M. officinalis* L. exhibited low activity against most of the bacteria with a zone of inhibition of

Table 3
The antimicrobial activities (zones of inhibition) of essential oil of *O. majorana* L. and its synergistic effect with antibiotics. mm.

Microorganisms	Essential oil	Standard antibiotic discs				Essential oil and standard antibiotic discs			
		TOB	TIC	IPM	G	TOB	TIC	IPM	G
E. coli	8.33 ± 1.52	20.33 ± 1.52	–	24.33 ± 0.57	20.33 ± 0.57	21.00 ± 0.00 A	10.00 ± 0.00 S	28.66 ± 0.57 A	19.00 ± 0.00 A
E. coli (ATCC 25921)	10.00 ± 1.00	22.33 ± 0.57	–	20.00 ± 0.00	22.66 ± 0.15	24.00 ± 0.57 A	11.66 ± 1.15 S	31.00 ± 0.15 S	23.33 ± 1.52 A
K. pneumoniae	9.66 ± 0.57	10.00 ± 0.00	20.66 ± 1.15	24.33 ± 0.57	22.00 ± 0.00	27.66 ± 1.15 S	27.00 ± 1.15 A	32.00 ± 0.57 S	32.00 ± 0.00 A
E. aerogenes	8.00 ± 1.00	10.00 ± 0.00	7.00 ± 0.00	17.66 ± 0.57	10.33 ± 0.57	14.00 ± 0.00 A	8.00 ± 0.57 I	16.00 ± 0.57 A	18.00 ± 0.57 Ad
S. aureus	9.66 ± 1.15	21.33 ± 0.57	–	–	22.33 ± 0.57	22.00 ± 0.57 A	–	–	20.00 ± 1.00 A
P. mirabilis	–	9.00 ± 0.00	19.00 ± 0.57	27.00 ± 0.00	8.33 ± 0.57	8.00 ± 0.00 A	17.33 ± 1.52 A	26.66 ± 1.15 A	–
P. putida	–	7.33 ± 0.57	14.00 ± 0.00	26.66 ± 0.57	–	9.33 ± 0.57 S	12.00 ± 0.00 A	28.00 ± 0.57 S	–
P. aeruginosa	–	21.33 ± 0.57	24.00 ± 0.00	24.00 ± 0.00	20.30 ± 0.57	9.00 ± 0.57 A	22.33 ± 1.52 A	26.00 ± 0.00 S	–
P. aeruginosa (ATCC 27853)	–	23.00 ± 1.00	23.66 ± 0.57	30.66 ± 1.15	18.66 ± 0.57	21.00 ± 1.00 A	8.00 ± 0.57 A	32.00 ± 1.15 S	10.00 ± 0.57 A
S. enteritidis	8.00 ± 1.00	21.33 ± 0.57	–	–	12.33 ± 0.57	20.00 ± 0.57 A	9.00 ± 0.00 S	29.00 ± 0.57 S	10.00 ± 0.00 A

Values are represented as mean ± SEM; I: Indifference; S: Synergy; A: Antagonism; Ad: Addition; TOB: Tobramycin; TIC: Ticarcillin; IPM: Imipenem; G: Gentamicin.

Table 4

The antimicrobial activities (zones of inhibition) of essential oil of *M. officinalis* L. and its synergistic effect with antibiotics. mm.

Microorganisms	Essential oil	Standard antibiotic discs				Essential oil and standard antibiotic discs			
		TOB	TIC	IPM	G	TOB	TIC	IPM	G
E. coli	8.66 ± 0.57	20.33 ± 1.52	–	24.33 ± 0.57	20.33 ± 0.57	26.33 ± 1.52 A	39.33 ± 0.57 S	26.00 ± 0.57 A	21.00 ± 0.57 A
E. coli (ATCC 25921)	8.00 ± 1.00	22.33 ± 0.57	–	20.00 ± 0.00	22.66 ± 0.15	21.00 ± 0.57 A	7.33 ± 0.57 A	28.00 ± 0.00 Ad	19.00 ± 0.15 A
K. pneumoniae	11.33 ± 0.57	10.00 ± 0.00	20.66 ± 1.15	24.33 ± 0.57	22.00 ± 0.00	24.00 ± 0.00 S	38.00 ± 1.15 S	9.33 ± 0.57 A	23.00 ± 0.00 A
E. aerogenes	8.00 ± 1.00	10.00 ± 0.00	7.00 ± 0.00	17.66 ± 0.57	10.33 ± 0.57	11.00 ± 0.00 A	–	17.00 ± 0.57 I	14.00 ± 2.00 A
S. aureus	8.33 ± 0.57	21.33 ± 0.57	–	–	22.33 ± 0.57	21.00 ± 0.57 I	–	–	19.00 ± 0.57 A
P. mirabilis	11.00 ± 1.00	9.00 ± 0.00	19.00 ± 0.57	27.00 ± 0.00	8.33 ± 0.57	26.00 ± 0.00 S	14.00 ± 0.57 A	9.00 ± 0.00 A	21.00 ± 1.73 S
P. putida	–	7.33 ± 0.57	14.00 ± 0.00	26.66 ± 0.57	–	8.00 ± 0.00 S	15.00 ± 0.00 S	24.00 ± 0.57 A	–
P. aeruginosa	–	21.33 ± 0.57	24.00 ± 0.00	24.00 ± 0.00	20.33 ± 0.57	21.00 ± 0.00 A	–	23.00 ± 1.15 A	19.33 ± 1.52 A
P. aeruginosa (ATCC 27853)	–	23.00 ± 1.00	23.66 ± 0.57	30.66 ± 1.15	18.66 ± 0.57	19.00 ± 0.00 A	21.00 ± 0.57 A	28.66 ± 1.15 A	15.00 ± 0.57 A
S. enteritidis	–	21.33 ± 0.57	–	–	12.33 ± 0.57	18.00 ± 0.57 A	–	24.00 ± 0.00 S	–

Values are represented as mean ± SEM; I: Indifference; S: Synergy; A: Antagonism; Ad: Addition; TOB: Tobramycin; TIC: Ticarcillin; IPM: Imipenem; G: Gentamicin.

Table 5

The antimicrobial activities (zones of inhibition) of essential oil of *C. coronarium* L. and its synergistic effect on antibiotics. mm.

Microorganisms	Essential oil	Standard antibiotic discs				Essential oil and standard antibiotic discs			
		TOB	TIC	IPM	G	TOB	TIC	IPM	G
E. coli	–	20.33 ± 1.52	–	24.33 ± 0.57	20.33 ± 0.57	21.66 ± 2.08 S	10.00 ± 0.57 S	26.00 ± 0.57 S	19.33 ± 0.57 A
E. coli (ATCC 25921)	–	22.33 ± 0.57	–	20.00 ± 0.00	22.66 ± 0.15	23.00 ± 0.57 A	11.33 ± 0.57 S	19.00 ± 0.00 A	27.00 ± 0.15 S
K. pneumoniae	9.66 ± 1.15	10.00 ± 0.00	20.66 ± 1.15	24.33 ± 0.57	22.00 ± 0.00	24.66 ± 2.08 S	28.00 ± 1.15 S	27.66 ± 1.15 A	18.00 ± 2.00 A
E. aerogenes	–	10.00 ± 0.00	7.00 ± 0.00	17.66 ± 0.57	10.33 ± 0.57	13.33 ± 0.57 S	–	14.00 ± 0.57 A	17.33 ± 2.51 S
S. aureus	–	21.33 ± 0.57	–	–	22.33 ± 0.57	22.66 ± 1.15 S	23.00 ± 0.00 S	26.00 ± 1.73 S	20.33 ± 0.57 A
P. mirabilis	–	9.00 ± 0.00	19.00 ± 0.57	27.00 ± 0.00	8.33 ± 0.57	10.00 ± 0.00 S	20.00 ± 0.57 S	25.00 ± 0.00 A	–
P. putida	–	7.33 ± 0.57	14.00 ± 0.00	26.66 ± 0.57	–	8.00 ± 0.57 S	7.00 ± 1.00 A	27.00 ± 0.57 S	–
P. aeruginosa	–	21.33 ± 0.57	24.00 ± 0.00	24.00 ± 0.00	20.33 ± 0.57	9.33 ± 0.57 A	19.66 ± 2.08 A	27.66 ± 1.15 S	–
P. aeruginosa (ATCC 27853)	–	23.00 ± 1.00	23.66 ± 0.57	30.66 ± 1.15	18.66 ± 0.57	21.00 ± 1.00 A	–	32.33 ± 1.52 S	12.66 ± 1.15 A
S. enteritidis	–	21.33 ± 0.57	–	–	12.33 ± 0.57	19.00 ± 0.57 A	14.00 ± 0.00 S	27.33 ± 0.57 S	10.66 ± 2.08 A

Values are represented as mean ± SEM; I: Indifference; S: Synergy; A: Antagonism; TOB: Tobramycin; TIC: Ticarcillin; IPM: Imipenem; G: Gentamicin.

(8.00 ± 1.00) mm to (11.00 ± 1.00) mm. And *M. officinalis* L. showed no activity against *P. putida*, *P. aeruginosa* (ATCC 27853), *S. enteritidis* and *P. aeruginosa* (Table 4).

Applying ticarcillin with the essential oil of *M. officinalis* L. had a synergistic effect on *E. coli*, *P. putida* and *K. pneumoniae* (Table 4), an antagonist effect was observed in *E. coli* (ATCC 25921), *P. mirabilis* and *P. aeruginosa*. No inhibitory effect was found for the rest of the bacteria. The synergistic effect was observed in gentamicin and imipenime against *P. mirabilis* and *S. enteritidis*, respectively. An additive effect was observed in *P. putida* and *P. aeruginosa*, while a synergistic effect was observed in *K. pneumoniae* and *P. mirabilis*. Also, indifferent effect on *S. aureus* and antagonist effect in other bacteria were observed when there was a combination between essential oil of *M. officinalis* L. and tobramycin.

3.5. C. coronarium L.

Essential oil of *C. coronarium* L. had no antibacterial activity against the tested bacteria except for *K. pneumoniae* having a small zone of inhibition of (9.66 ± 1.15) mm (Table 5).

The essential oil of *C. coronarium* L. exerted a synergistic effect against *K. pneumoniae*, *P. mirabilis*, *P. putida*, *S. aureus*, *E. aerogenes* and *E. coli*, and an antagonistic effect against *E. coli* (ATCC 25921), *P. aeruginosa* (ATCC 27853), *P. aeruginosa* and *S. enteritidis* when it was combined with tobramycin. The combination of ticarcillin with the essential oil of *C. coronarium* L. had a synergistic effect in *E. coli*, *E. coli* (ATCC 25921), *K. pneumoniae*, *P. mirabilis* and *S. auereus,* and an antagonistic effect in other bacteria. The application of imipenem with the essential oil of *C. coronarium* L. led to an antagonistic effect on *E. coli* (ATCC 25921), *E. aerogenes*, *K. pneumoniae* and *P. mirabilis* and a synergistic effect was observed in the other tested bacteria. A synergistic effect was observed in *E. coli* (ATCC 25921) and *E. aerogenes*, while the antagonistic effect was seen in the rest of the bacteria when there was a combination of the essential oil of *C. coronarium* L. with gentamicin.

4. Discussion

From this study, we can see that all antibiotics showed antibacterial activity against different bacterial strains, but at different levels. All the tested bacteria were more or less sensitive to five essential oils with the exception of *P. aeruginosa*. According to the literature *P. aeruginosa* is usually very sensitive to essential oils [20]. Khadir also showed that *P. aeruginosa* resisted the action of the essential oil of *Thymus lanceolatus* [21]. The essential oil of *T. willdenowii* Boiss showed significant antibacterial characters on the microorganisms tested with the exception of *P. putida* and *P. aeruginosa*. According to the literature, the essential oils of several species of thyme are

known for their antibacterial activities [22,23]. The application of *T. willdenowii* Boiss with ticarcillin, imipenem, gentamicin and tobramycin increased the antimicrobial activity of all tested antibiotics. However, an antagonistic effect was seen in some bacteria. Toroglu reported that the combination of essential oil of *Thymus eigii* and standard antibiotics *in vitro* led to an antagonistic effect on the tested bacteria [19].

The essential oil of *O. compactum* L. used in this study has antimicrobial activity against the tested strains with different diameters of inhibition zones from one strain to another with the exception of *P. aeruginosa*, *P. aeruginosa* (ATCC 27853), *P. mirabilis* and *P. putida*. Bouhdid *et al.* [24] also showed antimicrobial activity of essential oil of *O. compactum* L. against all tested bacteria except for *P. aeruginosa* which showed resistance. Our results were similar to the study. The application of *O. compactum* L. with ticarcillin, imipenem, gentamicin and tobramycin had a synergistic effect on all tested antibiotics against *P. putida*. While the antagonistic effect was also observed in some bacteria. Our result showed that the combined application of *O. majorana* L. with tested antibiotics led to the decrease in antimicrobial activity.

The essential oil *M. officinalis* L. showed relatively little effect against six bacterial strains with a zone of inhibition between (8.00 ± 1.00) mm and (11.33 ± 0.57) mm, but had no effect on *P. putida*, *P. aeruginosa* (ATCC 27853); *P. aeruginosa* and *S. enteritidis*. *M. officinalis* L. has been used internally and externally since ancient times for its sedative, digestive, appetizing, analgesic, antibacterial, antiviral and antioxidant activities [25]. The application of *M. officinalis* L. with ticarcillin, imipenem, gentamicin and tobramycin driven decreased antimicrobial activity against the bacteria, but a synergistic effect was also detected against some bacteria.

Essential oils of *C. coronarium* L. showed no antibacterial effect against all strains tested except *K. pneumoniaee* with very low sensitivity of (9.66 ± 1.15) mm.

According to Felice *et al.*, the essential oils of *C. coronarium* L. had no activity against *E. coli*, *S. aureus* and *P. aeruginosa* [26], which is consistent with the results of our study. The application of *C. coronarium* L. with ticarcillin, imipenem, gentamicin and tobramycin resulted in the decrease of the antimicrobial activity against the bacteria tested, but a synergistic effect was also detected against some bacteria.

This study indicates that the combination of essential oils of the five medicinal plants and the standard antibiotics has significant potential for the development of new antimicrobial treatment and reduction of drug resistance, which will permit to find the treatment of several diseases caused by microorganisms. From the results obtained, the essential oils acts in synergy with the tested standard antibiotics. This synergy could lead to new options for the treatment of infectious diseases and emerging drug resistance. There is a need for more studies on the molecular basis of the synergistic interaction to understand the synergistic mechanism that is fundamental for the development of pharmacological agents to treat bacterial infections using medicinal plants. Therefore, research should be focused in that direction to identify medicinal plants that have a synergistic behavior.

Conflict of interest statement

We declare that we have no conflict of interest.

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