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

Fadila Moussaoui*, Tajelmolk Alaoui

Laboratory of Environment and Health, University Moulay Ismail, Faculty of Sciences, BP11201, Meknes, Morocco

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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*

*Corresponding author: Fadila Moussaoui, Laboratory of Environment and Health, University Moulay Ismail, Faculty of Sciences, BP11201, Meknes, Morocco.
E-mail: moussaoui.fadila@gmail.com
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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.