In Vitro Synergistic Action of Certain Combinations of Gentamicin and Essential Oils

A. Rosato*[,1], M. Piarulli[1], F. Corbo[1], M. Muraglia[1], A. Carone[2], M.E. Vitali[3] and C. Vitali[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Bari, Bari, Italy;* [2]*Hospital Pharmacy, SS Annunziata Hospital, Taranto, Italy;* [3]*School of Specialization in Hygiene and Preventive Medicine, Faculty of Medicine, University of Bari, Bari, Italy*

Abstract: The aim of this study was to verify the existence of synergistic antibacterial effect between four essential oils (*Aniba rosaeodora, Melaleuca alternifolia, Origanum vulgare,* and *Pelargonium graveolens*) individually combined with the antibacterial drug Gentamicin. We investigated the effectiveness *in vitro* of the association of essential oil/Gentamicin, against fifteen different strains of Gram positive and Gram negative bacteria. The antibacterial effects of these oils in combination with Gentamicin were evaluated by using the MHB microdilution method, while gas chromatography (GC) and GC/Mass spectrometry were used to analyze the chemical composition of the oils. A synergistic interaction was observed against all tested strains with the associations between the essential oils *Aniba rosaeodora/Gentamicin* and *Pelargonium graveolens/Gentamicin*. In particular a very strong synergistic interaction was observed against *Acinetobacter baumannii ATCC 19606* (FIC index = 0.11). In contrast, the essential oils *Origanum vulgare* and *Melaleuca alternifolia* in association with Gentamicin were less effective on bacterial species growth. *In vitro* interaction can improve the antimicrobial effectiveness of the Gentamicin and may contribute to reduce its dose correlated to side effects.

Keywords: Antibacterial, synergism, *Aniba rosaeodora*, *Pelargonium graveolens*, Gentamicin, Gram negative, *Acinetobacter baumannii.*

INTRODUCTION

In the recent decades the antimicrobial research program focused its attention to search for newer compounds with potential antimicrobial activities, and worldwide spending on discovering new antimicrobial agents has increased in the last years. This real need is potentially ascribed both to the increasing emergence of bacterial resistance to antibiotic therapy and to newly emerging pathogens. Anyway, despite advances in antibacterial therapy, many problems remain to be solved for most available antimicrobial drugs. New source, especially the plant-derived antimicrobial compounds, are being investigated extensively in the recent years. In particular, a review of the recent literature revealed that among all natural products, plant essential oils are well known for their pharmacological activities, including antibacterial and antifungal activity, and may represent a promising source of new natural drugs [1-8]. Essential oils have been largely employed for their properties already observed in nature, i.e. for their antibacterial, antifungal and insecticidal activities. At the present time, approximately 3000 essential oils are known, 300 of which are commercially available in the food, agronomic, sanitary and pharmaceutical areas. As the chemical profile of the essential oil products depends on the type of extraction and on the number of molecules extracted, therefore, it is very difficult to establish a specific biological target involved in the mechanism of action. Probably, their biological profiles are the result of a synergism of all molecules present in the oil or they reflect only those of the main molecules present at the highest levels as could be revealed by gas chromatography. For the same reasons, no particular resistance or adaptation to essential oils has been described [9]. It is important to underline that some of them constitute effective alternatives or complements to synthetic compounds without showing the same secondary effects [10]. All these observations prompted us to start a research program with the aim to develop a new highly active antibacterial therapeutic combination of essential oils and synthetic antibacterial agents in order to provide better efficacy for combating various infections and drug resistance [11-15]. Between aminoglicosides Gentamicin is widely used for the treatment and prevention of life-threatening Gram-negative bacterial infections, but its several side effects limit its use.

The most relevant side effects are the toxicity to the sensory cells of the ear and nephrotoxicity. In addition, psychiatric symptoms related to Gentamicin (anorexia, confusion, depression, disorientation and visual hallucinations) can occur. Like all aminoglycosides, when Gentamicin is given orally, it is not systemically active. This is because it is not absorbed to any appreciable extent in the small intestine. It is administered intravenously, intramuscularly or topically to treat infections. It appears to be completely eliminated unchanged in the urine. Certain serious infections are conventionally treated with a prolonged dose of Gentamicin or other drugs. These include osteomyelitis, and endocarditis as well as serious and overwhelming infections.

In the present work we will discuss the *in vitro* association properties of Gentamicin and four essential oils extracted from *Aniba rosaedora, Melaleuca alternifolia, Origanum vulgare,* and *Pelargonium graveolens* against a large panel of Gram positive and Gram negative bacterial strains.

The antimicrobial activity of the four essential oils against different Gram positive and Gram negative bacterial strains and their synergistic effects when incorporated with Gentamicin were carried out by using the microdilution method.

*Address correspondence to this author at the Pharmaceutical Chemistry Department, Università degli Studi di Bari, via Orabona 4, 70125, Bari, Italy; Tel: +39 080 5442728; Fax +39 080 5442724;
E-mail: arosato@farmchim.uniba.it

0929-8673/10 $55.00+.00

EXPERIMENTAL SECTION

Material and Methods

The Eos (essential oils) EOs were purchased from Erbamea (Istrana, Treviso - Italy) and were obtained by steam distillation from *Melalueca alternifolia (Tea Tree), Origanum vulgare (Wild majoran), Aniba rosaeodora (Rosewood),* and *Pelargonium graveolens (Fragrant geranium)*. The composition of the essential oils was analyzed using a gas chromatographer and mass spectrometer (GC-MS) composed of an HP 6890 and an HP 5973, equipped with an HP-5 capillary column [16].

Bacterial Strains and Antimicrobial Testing

Several bacterial strains from American Type Culture Collection (ATCC, Rockville, MD, USA) were used as controls to test the antibacterial properties of the four essential oils and several of their components. Antibacterial activity was assessed against the following bacterial strains: *Bacillus cereus ATCC 7464, Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 6538p, Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 19433, Enterococcus faecalis ATCC 29212, Serratia marcescens ATCC 8100, Escherichia coli ATCC 35218, Escherichia coli ATCC 9735, Escherichia coli ATCC 25922, Yersinia enterocolitica ATCC 9610, Salmonella typhi ATCC 14028, Acinetobacter baumanni ATCC 19606, Klebsiella pneumonia ATCC 13883,* and lastly, *Pseudomonas aeruginosa ATCC 27853.* The bacterial species were cultured on Mueller Hinton agar (MHA, Oxoid), and each bacterial suspension was composed of 2-3 colonies of each strain taken from an MHA plate and dissolved in 2 mL of MHB (Mueller Hinton Broth). The resulting suspensions were diluted with 0.85% NaCl solution and then adjusted to $1x10^8$ CFU/mL (0.5 Mc Farland).

The MICs of the four essential oils and Gentamicin were determined by the broth microdilution method, according to CLSI (Clinical and Laboratory Standard Institute 2008) Protocol M7A6 guidelines [17].

In our experimental procedure we applied some modifications to CLSI Protocol M7A6. A final volume concentration of 40% (v/v) stock solution with Tween 80, 0.1% of each essential oil in ethanol (1: 2.5) was diluted 1: 20 in MHB to obtain a 2% (v/v) solution. Doubling dilutions of the essential oil from 2% to 0.25% for all four of the essential oils were prepared directly in 96 well microtitre trays in MHB, (0.06% to 0.23% for *Aniba rosaeodora*, 0.01% to 0.45% for *Pelargonium graveolens*, 0.18% to 1.5% (v/v) for *Melaleuca alternifolia*, and 0.03% to 0.25% for *Origanum vulgare*). After the addition of 0.02 mL of inoculum, the trays were incubated at 36 °C for 24 hours. The final concentration of Ethanol was 3% (v/v). The MHB medium well control 0.1% (v/v) Tween 80 and Ethanol 3%, (but without essential oil) was used as a positive growth control. The suspensions obtained were adjusted to 0.5 McFarland in agreement with CLSI Protocol M7A6. Plates were incubated at 36 °C for 24 hours.

MIC was defined as the lowest concentration that did not result in any visible growth of the bacterial strains compared to their growth in the control well [18]. As reported in Table **1** MIC values are given in mg/mL and μg/mL for essential oils and Gentamicin respectively. MIC determinations were realized in triplicate in three independent assays.

MIC data of the essential oils and Gentamicin were converted into Fractional Inhibitory Concentration (FIC) defined as ratio of the concentration of the antimicrobial in an inhibitory concentration with a second compound to the concentration of the antimicrobial by itself [19]. We decided to report the most interesting antimicrobial in Table **1**.

Microdilution Checkerboard Method

In the combination assays the checkerboard procedure described by White *et al*. [20] was followed to evaluate the synergistic action of the essential oils with Gentamicin. Twelve double serial dilutions of the four oils were prepared following the same method used to evaluate the MIC. Dilutions of the oil were prepared together with a series of double dilutions of the Gentamicin in the range of 16–0.25 μg/mL. This method was used to mix all the Gentamicin dilutions with the appropriate concentrations of oil so that a series of concentration combinations of the Gentamicin/particular oil being considered were obtained. In our experimental protocol the substance combinations were analyzed by calculating the FIC index (FICI) as follows: FIC of oil plus FIC of Gentamicin. Generally, FICI value was interpreted as : i) a synergistic effect when ≤ 0.5; ii) an additive or indifferent effect when > 0.5 and < 1; iii) an antagonistic effect when > 1 [19]. The concentrations prepared accounted for 40%, 20%, 10%, and 5% of the MIC value for the oil, and 25%, 12.5%, 6.25%, 3.12% of the MIC value for the antibiotic. The combination of the two components can be shown graphically in a Cartesian diagram by applying the isobole method. The non-interaction of the two components results in a straight line, whereas the occurrence of an interaction is shown by a concave isobole [21-25].

RESULTS

In this study four essential oils extracted from *Aniba rosaedora, Melaleuca alternifolia, Origanum vulgare,* and *Pelargonium graveolens* were used in association with Gentamicin. The effects of their combinations were evaluated on a large panel of Gram positive and Gram negative bacterial strains. The results for the interaction between the considered essential oils and Gentamicin are reported in Table **1** relatively to the most responsive bacterial strains. MICo (MIC of an individual sample) and MICc (MIC of an individual sample at the most effective combination) and FIC for *Origanum vulgare,* respectively, ranged from 0.3 to 0.6 mg/mL, 0.07 to 0.15 mg/mL, and 0.12 to 0.25 mg/mL. The MICo, MICc, and FIC of *Aniba rosaeodora* ranged from 0.25 to 2 mg/mL, 0.01 to 0.1 mg/mL, and 0.05 to 0.1 mg/mL, while the MICo, MICc, and FIC of *Melaleuca alternifolia* ranged from 1.7 to 13.8 mg/mL, 0.42 to 3.45 mg/mL, and 0.25 to 0.4 mg/mL. Furthermore, the MICo, MICc, and FIC of *Pelargonium graveolens* ranged from 0.12 to 1 mg/mL, 0.01 to 0.2 mg/mL, and 0.05 to 0.2 mg/mL respectively. Lastly, the MICo, MICc, and FIC of the Gentamicin ranged from 0.06 to 4 μg/mL, 0.01 to 1.6 μg /mL, and 0.03 to 0.4 μg /mL respectively.

The best results from the evaluated oils in association with Gentamicin were obtained from *Aniba rosaedora*, and

Table 1. Essential Oils and Gentamicin - Fractional Inhibitory Concentration (FIC) and FIC Indices

	O. vulgare (mg/ml)				A. rosaeodora (mg/ml)				M. alternifolia (mg/ml)				P. graveolens (mg/ml)			
	MICo	**MICc**	**FIC**	**FICI**	**MICo**	**MICc**	**FIC**	**FICI**	**MICo**	**MICc**	**FIC**	**FICI**	**MICo**	**MICc**	**FIC**	**FICI**
Bacillus cereus ATCC 7464																
Essential oil (mg/ml)	0.60	0.15	0.25	0.28	0.25	0.01	0.05	0.30	1.70	0.68	0.40	0.52	0.50	0.025	0.05	0.30
Gentamicin (μg/ml)	0.50	0.01	0.03		0.50	0.12	0.25		0.50	0.06	0.12		0.50	0.125	0.25	
Bacillus subtilis ATCC 6633																
Essential oil (mg/ml)	0.60	0.07	0.12	0.33	0.50	0.05	0.10	0.34	6.90	1.70	0.25	0.50	0.50	0.05	0.10	0.34
Gentamicin (μg/ml)	0.25	0.05	0.20		0.25	0.06	0.24		0.25	0.06	0.25		0.25	0.06	0.24	
Staphylococcus aureus ATCC 29213																
Essential oil (mg/ml)	0.60	0.15	0.25	0.31	0.50	0.02	0.05	0.30	13.8	3.45	0.25	0.50	0.24	0,06	0.25	0.28
Gentamicin (μg/ml)	0.50	0.03	0.06		0.50	0.12	0.25		0.50	0.12	0.25		0.50	0.01	0.03	
Staphylococcus aureus ATCC 6538p																
Essential oil (mg/ml)	0.60	0.15	0.25	0.51	0.25	0.01	0.05	0.30	6.90	1.72	0.25	0.49	0.25	0.01	0.05	0.35
Gentamicin (μg/ml)	0.06	0.02	0.26		0.06	0.01	0.25		0.25	0.06	0.24		0.12	0.04	0.30	
Escherichia coli ATCC 25922																
Essential oil (mg/ml)	0.60	0.15	0.25	0.65	0.50	0.05	0.10	0.35	6.90	1.72	0.25	0.49	1.00	0.05	0.05	0.30
Gentamicin (μg/ml)	0.50	0.20	0.40		0.50	0.12	0.25		0.50	0.12	0.24		0.50	0.12	0.25	
Acinetobacter baumannii ATCC19606																
Essential oil (mg/ml)	0.60	0.15	0.25	0.65	0.25	0.01	0.05	0.11	3.40	0.85	0.25	0.50	0.5	0.02	0.05	0.11
Gentamicin (μg/ml)	4.00	1.60	0.40		4.00	0.24	0.06		4.00	1.00	0.25		4.00	0.24	0.06	
Serratia marcescens ATCC 8100																
Essential oil (mg/ml)	0.60	0.15	0.25	0.65	2.00	0.10	0.05	0.30	6.90	1.72	0.25	0.49	1.00	0.20	0.20	0.45
Gentamicin (μg/ml)	0.25	0.10	0.40		0.50	0.12	0.25		0.25	0.06	0.24		0.50	0.12	0.25	
Yersinia enterocolitica ATCC 9610																
Essential oil (mg/ml)	0.30	0.07	0.25	0.63	2.00	0.10	0.05	0.11	1.70	0.42	0.25	0.49	0.50	0.05	0.10	0.22
Gentamicin (μg/ml)	0.25	0.01	0.38		0.25	0.01	0.06		0.25	0.06	0.24		0.25	0.03	0.12	

MIC_o, =MIC of an individual sample, MIC_c,= MIC of an individual sample at the most effective combination; FIC= Fractional Inhibitory Concentration (see text); FICI = FIC of oil + FIC of Gentamicin.

Pelargonium graveolens. In particular, the MIC value of Gentamicin is reduced from 4 to 0.24 μg /mL for *Acinetobacter baumanii* ATCC 19606, a very difficult Gram negative bacillus to treat with Gentamicin alone. The FIC indexes (FICI = 0.11 for both associations) of Gentamicin/*Aniba rosaedora* and Gentamicin/*Pelargonium graveolens* indicate, in fact, a very strong synergistic mode of action. Even if the results of the associations *Aniba rosaedora*, and *Pelargonium graveolens* with Gentamicin, relatively to MICs of the other bacterial strains reported in Table **1** are less pronounced respect to *Acinetobacter baumanii* ATCC 19606, these mixtures show good synergistic effects on all bacterial strains studied (FICI ranged between 0.22-0.30). Furthermore, a significant effect is obtained with association of Gentamicin/*Origanum vulgare* with some Gram positive bacillus, as *Bacillus cereus* ATCC 7464 (FICI 0.28),

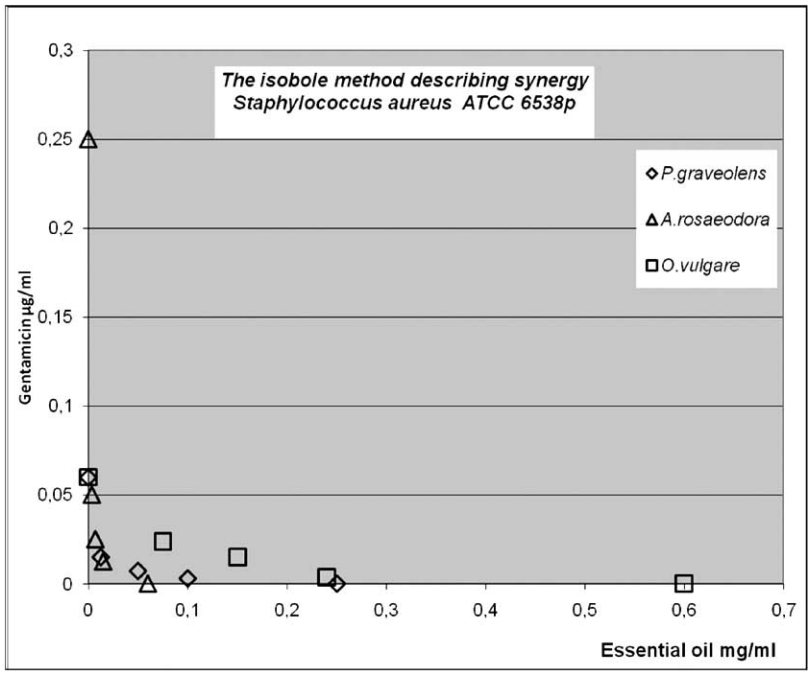

Fig. (1). Isobole curve revealing the synergistic effect of combining *Pelargonium graveolens*, *Aniba rosaeodora*, *Origanum vulgare*, with Gentamicin in inhibiting *Staphylococcus aureus ATCC 6538p*. (Inoculum size 1-2 x 10^8 CFU/mL).

Bacillus subtilis ATCC 6633 (FICI 0.33) and *Staphylococcus aureus* ATCC 29213 (FICI 0.31). Finally, our data demonstrated that the obtained mixture act with good synergistic activity against all tested Gram positive strains. No synergistic effect was observed in the combination experiments of *Melaleuca alternifolia* and Gentamicin against all studied bacterial strains (FICI 0.5). Figs. (**1**, **2** and **3**) describe the synergistic interaction between various oils and the antibiotic Gentamicin [25, 26]. The combination of the two components is shown by most common graphic representation called isobologram (iso-effect curve). It is worthy to note that an isobologram is constructed by outlining on the x and y axes, which are the inhibitory doses of two agents, for a given effect. If the effect is additive, the totaled doses are proportional to the effect, which is verified by a shift towards the straight line on the graph, which connects two doses with equal effects, a linear isobol, when the agents are not synergic. When the curve of the isobol moves towards the origin (concave line) this indicates that the agents in the mixture are synergic, and when the opposite occurs (convex line) they present antagonism. In the other words, the same biological effects of the agents in isolation is obtained at lower (or higher) doses of the mixture [27]. Because the synergistic effect of Gentamicin/*Melaleuca alternifolia* is not substantial, it is not reported on the graphs. Note how the isobole points of the *Aniba rosaeodora* and the *Pelargonium graveolens* (Fig. (**1**)) are closer to the origin of the axes than the other two oils. The graphs indicate strong synergism against *Staphylococcus aureus* ATCC 6538p by the obviously deviating points to the left [28] Fig. (**2**) describes another interesting aspect of our work, the strongest synergistic efficacy of the mixture Gentamicin/*Aniba rosaeodora* or *Pelargonium graveolens* essential oils against Gram negative bacteria *Acinetobacter baumannii* ATCC 19606 with a FIC index equal to 0.11. Also, Fig. (**3**) shows strong synergistic effect against *Escherichia coli* ATCC 25922 with a FIC index equal to 0.35 that justify the interaction between the essential oil and Gentamicin. Figs. (**1**, **2** and **3**) show that *Aniba rosaeodora* and *Pelargonium graveolens* essential oils have a high level of efficacy for their antibacterial ability. The synergistic antibacterial action demonstrated by combining either *Melaleuca alternifolia* (data not shown) or *Origanum vulgare* with Gentamicin was less pronounced or absent (*Melaleuca alternifolia*). To identify the composition of the tested oils, we analyzed the oils derived from the steam distillation of the four plant species by GC-MS [29]. The composition of these oils differs for the presence of some components as shown in Table **2**. The terpenalcohol fraction was the most prominent component in *Aniba rosaeodora* (74%) and *Pelargonium graveolens* (67%) essential oils, while this component is less present in *Melaleuca alternifolia* (38%) essential oil or completely absent in *Origanum vulgare* essential oil. Table **2** shows more phenolic compounds in *Origanum vulgare*, and more hydrocarbons in *Melaleuca alternifolia*.

Table **1** reveals how the Gentamicin/*Origanum vulgare* association has an indifferent and non synergistic effect against five of the eight strains studied, and the Gentamicin/*Melaleuca alternifolia* association has an additive and non synergistic effect for the considered bacteria. In contrast, the effect of the *Aniba rosaeodora* and *Pelargonium graveolens* associations is always synergistic on all of the considered strains with an extreme FICI value of 0.11 for the Gram negative bacteria *Acinetobacter baumanni* ATCC 19606.

FICI values reported in Table **1** for the association of *Aniba rosaeodora* and *Pelargonium graveolens* with Gentamicin, are respectively in the range of 0.11–0.34 for all the studied bacterial strains. Furthermore, MIC Gentamicin is

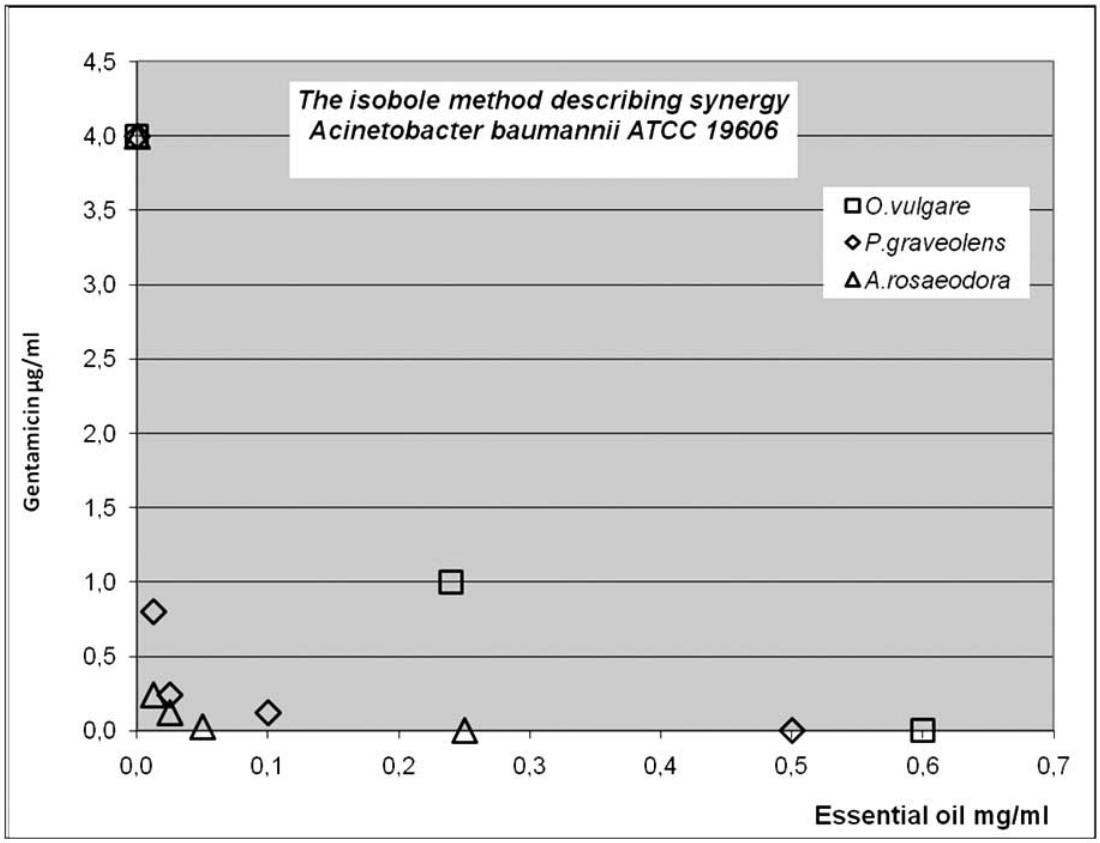

Fig. (2). Isobole curve revealing the synergistic effect of combining *Pelargonium graveolans*, *Aniba rosaeodora*, *Origanum vulgare*, with Gentamicin in inhibiting *Acinetobacter baumanii ATCC 19606* (Inoculum size 1-2 x 10^8 CFU/mL).

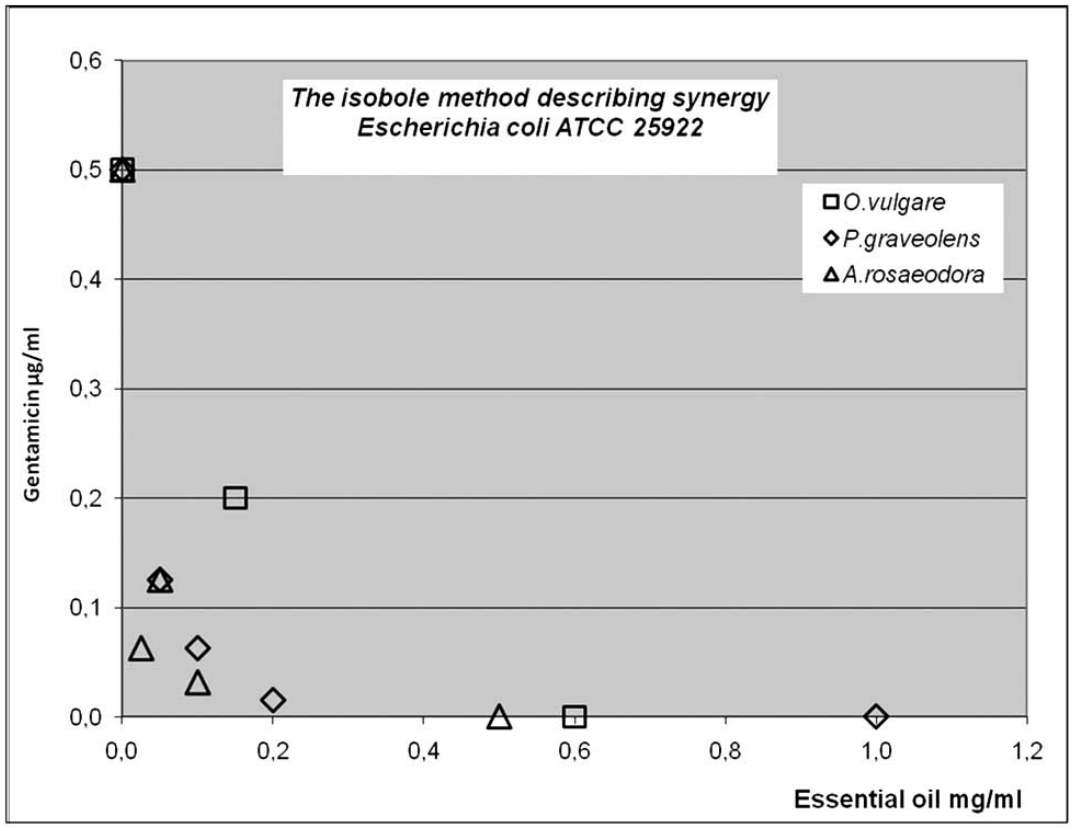

Fig. (3). Isobole curve revealing the synergistic effect of combining *Pelargonium graveolans*, *Aniba rosaeodora*, *Origanum vulgare*, with Gentamicin in inhibiting *Escherichia coli ATCC 25922*. (Inoculum size 1-2 x 10^8 CFU/mL).

Table 2. Chemical Composition of Studied Essential Oils (GC-MS). Area %

Essential oil	Area %
Melaleuca alternifolia	
Total Hydrocarbons	58
γ - Terpinene	16,3
2 - Carene	8,3
Eucalyptol	4,5
Total Terpenalcohols	38
Terpinen – 4 -ol	30,3
Caryophyllene	15
α - Terpineol	4,4
Total Phenols	0,17
Thymol	0,17
Pelargonium graveolens	
Total Hydrocarbons	30,5
Epizonalene	6
Caryophyllene	1,13
Total Terpenalcohols	67
Linalool	8,81
Citronellol	47,3
Geraniol	9,1
Origanum vulgare	
Total Hydrocarbons	37
α Pinene	5,1
Cymene	25
Eucalyptol	2,8
Total Phenols	63
Cymenol	58,6
Thymol	3,7
Aniba rosaedora	
Total Hydrocarbons	26,5
α Pinene	3,2
Cymene	4,1
Lymonen	19,2
Total Terpenalcohols	73,9
Linalool	60,1
Geraniol	7,8

remarkably decreased if it is combined with *Aniba rosaeodora* and *Pelargonium graveolens*. In detail, we have on average a reduction about 7 times for Gentamicin and 17 times for *Aniba rosaeodora* essential oil, and 14 times for Gentamicin and 8 times for *Pelargonium graveolens* essential oil. Particularly interesting is the high synergistic effect recorded for the two cited oils with Gentamicin against Gram negative bacteria so as *Serratia marcescens* ATCC 8100, *Escherichia coli* ATCC 25922, *Yersinia entrocolitica* ATCC 9610, *Acinetobacter baumannii* ATCC 19606.

DISCUSSION

Essential oils are very complex natural mixtures which contain about 20-60 components at quite different concentrations. The components include two groups of distinct biosynthetical origin: the main group is composed of terpenes and terpenoids and the other aromatic and aliphatic constituents are all characterized by low molecular weight [30]. Regarding their biological properties they are the results of a synergism of all molecules or reflect only those of the main molecules present at the highest levels according to gas chromatographical analysis. In this study we report the antimicrobial activity of Gentamicin evaluated in association with four essential oils extracted by *Aniba rosaedora*, *Melaleuca alternifolia*, *Origanum vulgare*, and *Pelargonium graveolens* on a large panel of Gram positive and Gram negative bacterial strains.

The combination of Gentamicin/*Pelargonium graveolens* and Gentamicin/*Aniba rosaeodora* administered against the bacterial strains under consideration is likely to reduce Gentamicin minimum effect dose. In fact, as reported in Table **1**, Gentamicin MIC and FICI in combination with the above cited essential oils are particularly interesting against the studied Gram negative bacterial strains. It is important to underline the strong synergy observed between Gentamicin and *Aniba rosaeodora* against the Gram negative *Acinetobacter baumannii*. In detail, Gentamicin MICc is much lower than those normally required to show the direct inhibition of bacteria growth (MICc: 0.24 μg/mL *vs* MICo: 4 μg/mL).

To elucidate the mechanism of action on the basis of the synergism of Gentamicin/essential oils and in particular Gentamicin/*Pelargonium graveolens* and Gentamicin/*Aniba rosaeodora* is very difficult. For this purpose different hypothesis should be taken into account. All interactions between antimicrobial compounds can alter effectiveness and synergistic or antagonistic relationships may result in competition for possible primary target [31]. On the other hand, a synergistic multi-target effect could occur by involving enzymes, substrates, metabolites and proteins, receptors, ion channels, transport protein, ribosomes, DNA/RNA and physicochemical mechanism [32].

Alternatively, interaction between different compounds may lead to changes in structural conformation, thus resulting in the reduction of the inhibitory activity [33].

Because there could be a lot of potential mechanism of action on the basis of a synergistic effect at the beginning of this research program, thus we were unable to investigate the exact mechanism of action. We hypothesized to rationalize the contribution of each essential oil in association with Gentamicin by evaluating different chemical compositions of the cited essential oils as reported by gas chromatographic analysis (Table **2**). As suggested by Table **2**, the antimicrobial activity and so the synergistic effect of *Pelargonium graveolens* and *Aniba rosaeodora*, could be attributed to the high percentage of terpenalcohols that former represent the

major component of both oils. At this purpose, recent literature studies suggest that monoterpenes contained in essential oils interact with model membranes and their antimicrobial effect may be ascribed to a damage of the microbial lipid membrane fraction [34]. In relation to the lipid composition and the net surface charge of the microbial membrane, the Gram negative outer membrane has a strong negative charge conferred by the lipopolisaccharide.

All these considerations prompted us to hypothesize that the high level of terpenalcohols in *Pelargonium graveolens* (63%) and *Aniba rosaeodora* (73%) favours the Gentamicin mechanism of action whose main effect is to interrupt the protein synthesis by binding the 30S subunit of bacteria ribosome.

In conclusion, here we have shown that *in vitro* association of Gentamicin/ *Pelargonium graveolens* and Gentamicin/*Aniba rosaeodora* is particularly interesting against Gram negative bacteria. In detail, this association was found to produce a substantial Gentamicin MIC reduction against *Acinetobacter baumannii*, a Gram negative bacteria whose pharmacological treatment is very difficult nowadays. Thus, these important results may help the formulation of new topical agents for the cure of infections caused by the Gram negative bacteria.

Finally, we suppose that this work could provide a platform for active researchers to perform further experimental procedures to provide greater details about the mechanism of action of the synergism so as to plan clinical trial to confirm these *in vitro* results.

REFERENCES

[1] Dorman, H.J.; Deans, S.G. Anti-microbial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.*, **2000**, *88*, 308-316.

[2] Lis-Balchin, M. *Aromatherapy Science - A guide for healthcare professionals*, Pharmaceutical Press: London, **2006**.

[3] Avato, P.; Raffo, F.; Guglielmi G.; Vitali, C.; Rosato, A. Extracts from St. John's Wort and their antimicrobial activity. *Phytother. Res.*, **2004**, *18*, 230-232

[4] Avato, P.; Bucci, R.; Tava, A.; Vitali, C.; Rosato, A.; Bialy, Z.; Jurzysta M. Antimicrobial activity of saponins from *Medicago spp*.: structure-activity relationship. *Phytother. Res*., **2006**, *20(6)*, 454-457.

[5] Bidlack, W.R.; Omaye, S.T.; Meskin, M.S.; Topham, D. Phytochemicals as Bioactive Agents, Lancaster, Technomic Publishing Company: UK, **2000**, pp. 106-110.

[6] Rosato, A.; Vitali, C.; Gallo, D.; Balenzano, L.; Mallamaci, R. The inhibition of Candida species by selected essential oils and their synergism with *Amphotericin B. Phytomedicine,* **2008**, *15*, 635-638.

[7] Rosato, A.; Vitali, C.; Piarulli, M.; Mazzotta, E.; Mallamaci, R. *In vitro* synergic efficacy of the combination *Nystatin* essential oils against some Candida species. *Phytomedicine,* **2009**, *16*, 972-975.

[8] Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils - A review. *Food Chem. Toxicol.*, **2008**, *46*, 446-475.

[9] Silva, J.; Abebe, W.; Sousa, S.M.; Duarte, V.G.; Machado, M.I.L.; Matos, F.J.A. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. *J. Ethnopharmacol*., **2003**, *89*, 277-283

[10] Carson, C.F.; Riley, T.V. Non-antibiotic therapies for infectious diseases. *Commun. Dis. Intell.*, **2003**, S143-S146.

[11] Cox, S.D.; Mann, M.; Markham, J.L.; Bell, J.E.; Gustafson, J.E.; Warmington, J.R.; Wyllie, S.G. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J. Appl. Microbiol.*, **2000**, *88*, 170-175.

[12] Shin, S. ; Kang, C.A. Antifungal activity of the essential oil of *Agastache rugosa Kuntze* and its synergism with Ketoconazole. *Lett. Appl. Microbiol.*, **2003**, *36*, 111-115.

[13] Shin, S.W.; Lim, S. Antifungal effects of herbal essential oils and in combination with ketoconazolo against Trichopyton spp. *J. Appl. Microbiol.*, **2004**, *97*, 1289-1296.

[14] Yoon, S.Y.; Eo, S.K.; Lee, D.K.; Han, S.S. Antimicrobial activity of Ganoderma lucidum extract alone and in combination with some antibiotics. *Arch. Pharm. Res.*, **1994**, *6*, 438-442.

[15] Hammer, K.A.; Carson, C.F.; Riley, T.V. Antimicrobial activity of essential oils and other plant extract. *J. Appl. Microbiol.*, **1999**, *86*, 985-990.

[16] Adams, R.P. *Identification of Essential Oils by Trap Mass Spectrometry*, Allured Publish Corporation: Carol Stream, IL, **2007**.

[17] Clinical Laboratory Standards Institute Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard: M7-A6, vol. *23* - n°2, CLSI: Villanova, PA **2002**.

[18] European Committee for Antimicrobial Susceptibility Testing (EUCAST) *Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents, in* Eucast definitive document E.Def 1.2, European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Basel (SW), **2000**; vol. *6*, pp. 503-508.

[19] Te Dorsthorst, D.T.A.; Verweij, P.E.; Meletiadis, J.; Bergevoet, M.; Punt, N.C.; Meis, J.F.G.M.; Mouton, J.W. *In vitro* interaction of flucytosine combined with amphotericin B or fluconazole against thirty-five yeast isolates determined by both the fractional inhibitory concentration index and the response surface approach. *Antimicrob. Agents Chemother.*, **2002**, *46*, 2982-2989.

[20] White, R.L.; Burgess, D.S.; Manduru, M.; Bosso, J.A. Comparison of three different *in vitro* methods of detecting synergy: time-kill, checkerboard, and E test. *Antimicrob. Agents Chemother.*, **1996**, *40*, 1914-1918.

[21] Bapela, N.B.; Lall, N.; Fourie, S.G.; Franzblau, S.G.; Van Rensburg C.E.J. Activity of 7-methyljuglone in combination with antitubercolous drugs against *Mycobacterium tubercolosis*. *Phytomedicine*, **2006**, *13*, 630-635.

[22] Cassella, S.; Cassella, J. and Smith, I. Synergistic antifungal activity of tea tree (*Melaleuca alternifolia*) and lavender (*Lavandula angustifolia*) essential oil against dermatophyte infection. *International Journal of Aromatheraphy*, **2002**, *12*, 2-15.

[23] Eloff, J.N. Quantification the bioactivity of plant extracts during screening and bioassay guided fractionation. *Phytomedicine,* **2004**, *11(4)*, 370-1.

[24] Giamperi, L.; Fraternale, D.; Ricci, D. The *in vitro* action of essential oils on different organisms. *J. Essen. Oil Res.*, **2002**, *14*, 312-318.

[25] Berenbaum, M. What is synergy? *Pharmacology*, **1989**, *41*, 93-141.

[26] Williamson, E.M. Synergy and other interactions in phytomedicines. *Phytomedicine,* **2001**, *8* (5), 401-409.

[27] Biavatti, M.W. Synergy: an old wisdom, a new paradigm for pharmacotherapy. *Br. J. Pharm. Sci.*, **2009**, *45* (3), 371-378.

[28] Rosato, A.; Vitali C.; De Laurentis, N.; Armenise, M. and Milillo, M.A. Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. *Phytomedicine,* **2007**,*14*, 727-732.

[29] Hethelyi, E.; Koczka, I.; Tetenyi, P. Phytochemical and antimicrobial analysis of essential oils. *Herba Hungarian*, **1989**, *28*, 1-2.

[30] Croteau, R.; Kutchan, T.M.; Lewis, N.G. Natural products (secondary metabolites). In: Buchanan, B., Gruissem, W., Jones, R. (Eds.), *Biochemistry and molecular biology of Plants. Am. Soc. Plant Phiysiol.*, **2000**.

[31] Mandalari, G.; Bennett, R.N.; Bisignano, G.; Trombetta, D.; Saija, A.; fauldus, C.B.; Gasson, M.J.; Narbad, A. Antimicrobial activity of flavoids extracted from bergamot (*Citrus bergamia* Risso) peel, a by-product from the essential industry. *J. Appl. Microbiol*., **2007**, *103*, 2056-2064.

[32] Wagner, H.; Ulrich-Merzenich, G. Synergy research: approaching a new generation of phytopharmaceuticals. *Phytomedicine*, **2009**, *16*, 97-110.

[33] Lis-Balchim, M.; Deans, S.G.; Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. Appl. Bacteriol.* **1997**, *82*, 759-762.

[34] Trombetta, D.; Castelli, F.; Sarpietro, M.G.; Venuti, V.; Cristiani, M.; Daniele, C.; Saija, A.; Mazzanti, G.; Bisignano, G. Mechanisms of antibacterial action of three monoterpenes. *Antimicrob. Agents Chemother.*, **2005**, *49*, 2474-2478.

Received: April 05, 2010 **Revised: July 08, 2010** **Accepted: July 10, 2010**