A novel interpretation of the Fractional Inhibitory Concentration Index: The case Origanum vulgare L. and Leptospermum scoparium J. R. et G. Forst essential oils against Staphylococcus aureus strains

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ABSTRACT

Origanum vulgare (oregano) and Leptospermum scoparium (manuka) were traditionally employed as natural remedies for infected wounds and skin injuries where Staphylococcus aureus is mainly involved.

The first aim of this study was to investigate oregano and manuka essential oils (EOs) chemical compositions and evaluate their antibacterial activity (MIC, Minimum Inhibitory Concentration) against fourteen

S. aureus wild strains. The second aim was to evaluate the antibacterial activities of oregano and manuka EOs mixed in different combination (FIC, Fractional Inhibitory Concentration) with an improved chequerboard technique. This allowed to avoid the usual uncertainty in the determination of MIC and FIC values and to obtain a more precise interpretation of FIC indexes (FICIs). Moreover, FICIs were discussed on the basis of a novel interpretation method to evaluate the synergistic/antagonistic effect of EOs mixtures.

The most representative compounds in oregano EO were Carvacrol (65.93%), p-Cymene (9.33%) and γ-Terpinene (5.25%), while in manuka EO were Leptospermone (31.65%), cis-Calamenene (15.93%) and Flavesone (6.92%). EOs presented MIC values ranging from 1:2048 to 1:4096 v/v and FIC values ranging from 0.125 to 1.

According to our interpretation, a synergistic effect (34.68%), a commutative effect (15.32%) and an indifferent effect (50.00%) and no antagonistic effect were observed. Conversely, according to two previously proposed FICI interpretation models, 1.80% synergistic effect could be observed and, respectively, 98.20% indifferent effect or 48.20% additive effect and 50.00% indifferent effect.

As practical results, oregano and manuka EOs may be an effective alternative to chemotherapic drugs in staphylococcal infections and useful tools to enhance food security.

1. Introduction

During the last years, essential oils (EOs), plant extracts, herbs and other vegetable derivate products were widely studied for their antimicrobial activity (Casella et al., 2013; Kazemi, 2015; Marques et al., 2015).

EOs have been used since ancient times for their flavour and preservation properties (Burt, 2004). EOs production by distilla- tion was first carried out around 2000 years ago in the East and Mediterranean areas (Guenther, 1948) and successively perfected

by Arabs in the 9th century (Bauer et al., 2001). Similarly, EOs (Melaleuca alternifolia and other Myrtaceae) seemed to be used in Australia by the native inhabitants before the European colonisa- tion (Carson, 1993). Currently, EOs are widely used and studied in human medicine for their properties as anticarcinogenic, antinoci- ceptive, antiphlogistic, antiviral, and antibacterial properties (Baser and Buchbauer, 2009).

In the last years, scientific interest in the EOs has increased due to their potential employment as products in food industries and as well as alternative treatments to several animal diseases (Bajpai et al., 2012; Fratini et al., 2014).

Origanum vulgare L. (oregano) is an aromatic herb belong- ing to the Lamiaceae family, widespread in Mediterranean area. Since the time of Hippocrates, the 5th century B.C., oregano was

recommended for the treatment of different pathological condi- tions as angina, inflammation around wounds, menstrual flows, pleurisy and pulmonary apoplexy (Pollio et al., 2008). Tradition- ally, oregano was employed in folk medicine to treat respiratory disorders, dyspepsia, painful menstruation, rheumatoid arthritis, scrofulosis, urinary tract disorders and as remedy against wounds and burns (Altiok et al., 2010; Gruenwald et al., 2000). Generally, the main constituents of oregano EO are represented by monoter- penes (Carvacrol, Thymol, γ-Terpinene and p-Cymene), depending on the chemotype; additionally, low amounts of other components are also present in relation to plant species, such as Caryophyllene, Linalool, Terpinene-4-ol and Germacrene (Gruenwald et al., 2000). Leptospermum scoparium J. R. et G. Forst (manuka) is a small tree or shrub belonging to the Myrtaceae family, widely dis- tributed in New Zealand and Australia (Porter and Wilkins, 1999). Since the beginning of the past century, bark, leaves, sap and seed capsules were used in medical preparations and beverages (Best, 1905; Brooker et al., 1981); moreover, manuka was usually employed in traditional Maori remedies (Riley, 1994). Nowadays, manuka is mainly cultivated in New Zealand for honey produc- tion; during the season 2012 was estimated that the 80–90% of the 120$ millions of honey exportation was manuka honey (Ministry for Primary Industries, 2013). Manuka EO is less known than manuka honey, even though the EO is worldwide employed for its activity against Gram positive bacteria, including antibiotic resis- tant strains (Douglas et al., 2004). Generally, the most important manuka EO compounds are represented by Leptospermone, iso-Leptospermone and Flavesone, the main triketone constituents (Douglas et al., 2004; Porter and Wilkins, 1999).

It has been shown that Staphylococcus aureus colonize from 30 to 50% of healthy adults and it is able to rapidly infect skin lesions with a consequential inflammatory process (Hauser et al., 1985; Kluytmans et al., 1995; Lowy, 1998). Moreover, S. aureus is associated with bovine mastitis with a high tendency to become persistent and may develop in a chronic form (Gill et al., 2006). Fur- thermore, enterotoxins producer strains S. aureus represent one of the most important agent involved in food-borne diseases, mainly due to raw milk and its products consumption (Pedonese et al., 2014).

Both oregano and manuka EOs are studied for their antibacterial activity against several pathogenic and saprophytic microorgan- isms (Burt, 2004; Elgayyar et al., 2001); in particular, from recent scientific literature, they show a remarkable inhibitory effect against S. aureus (Dal Pozzo et al., 2011; Fratini et al., 2014; Kumar et al., 2014). Usually, the antibacterial activity assays of EOs are car- ried out employing culture collection strains or a limited number of isolates; consequently, the current knowledge on the susceptibility of wild bacterial strains or EOs potential activity is limited.

In addition, many studies were carried out on several EOs to screen their antimicrobial activity, but few surveys have focused on their effect in combination (Bassolé and Juliani, 2012; Fratini et al., 2014).

Fractional Inhibitory Concentration method (FIC) was used since the early eighties on drugs with different practical techniques and several discordant result interpretations (EUCAST, 2000; Greco et al., 1995; Hall et al., 1983; Odds, 2003).

The first aim of our research was to investigate the chemical composition of O. vulgare and L. scoparium EOs and to test their antimicrobial activity (MIC; Minimum Inhibitory Concentration) against S. aureus wild strains; the second aim was to evaluate the antimicrobial activity of their mixtures (FIC) in order to detect syn- ergistic or antagonistic effects against S. aureus and discuss these effects (FICI, FIC index) on the basis of an improved chequerboard technique.

2. Materials and methods

2.1. Essential oils

Essential oils (EOs) of O. vulgare L. (Ov) and L. scoparium J. R. et

G. Forst (Ls) were purchased from FLORA®(Pisa, Italy). According to the certificates supplied by the company, Ov and Ls originated from Italy and New Zealand, respectively, and both EOs were extracted by steam distillation from the leaves of the plants. A chemical char- acterization of the two oils was carried out in the laboratory of Department of Pharmacy (University of Pisa) by GC–MS analyses. EOs were stored at 4 2 ◦C in the dark and were subjected to micro- bial analysis for quality control before their employment in the following tests.

2.2. GC and GC–MS analyses

GC–EIMS (Gas Chromatography–Electron Impact Mass Spec- trometry) analyses were performed with a Varian CP-3800 gas chromatograph equipped with a HP-5 capillary column (30 m 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures at 220 and 240 ◦C respectively; oven temperature was programmed from 60 ◦C to 240 ◦C at 3 ◦C min−1;

carrier gas helium at 1 ml min−1; injection of 0.2 μl (10% hex- ane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their Linear Retention Indices (LRIs) relative to the series of n-hydrocarbons, and by computer match- ing against commercial (NIST 98 and ADAMS 95) and home-made library mass spectra built up from pure substances and compo- nents of known essential oils and MS literature data, as previously described (Flamini et al., 2014).

2.3. Bacterial strains

Fourteen S. aureus wild strains belonging to the strains collection of the Department of Veterinary Science of Pisa University (named A, B, C, D, E, F, G, H, I, J, K, L, M and N), were previously isolated from bovine bulk tank milk and genotyped according to Tambic et al. (1997). The strains were stored at 80 ◦C in a glycerol suspension until their use.

Before MIC and FIC determinations, the strains were cultured in BHI (Brain Hearth Infusion, Oxoid, Milan, Italy) broth for 24 h at

37 ◦C in aerobic conditions.

2.4. Determination of MIC0

EOs MIC0 values for each strains were determined using twofold serial microdilution method according to Wiegand et al. (2008) with slight modifications. 190 µl of BHI was distributed in each well of a 96-well polypropylene microtiter plate, except for the first column. EO dilution was prepared in BHI supplemented with Dimethyl sulfoxide (DMSO) as a solvent to a final ratio of 1:3:4 (v/v). 380 µl of EO dilution was dispensed in the first well of each strain row and twofold dilution series were performed to the 10th well. From the 10th wells of each row 190 µl were discarded. Then, 10 µl of a bacterial suspension adjusted to the point 0.5 of the McFar land standard turbidity scale (approximately 1.5*108 CFU per ml) were added to each well of the respective strain row to reach a final volume of 200 µl. The 11th and 12th wells were employed for the positive and negative controls, respectively. Microplates were incubated at 37 ◦C for 24 h in a humid chamber. EOs MIC determi- nations were performed in triplicate and for each strain the mode was determined (MIC0).

2.5. Determination of FIC and FIC index (FICI)

The combined effect of EOs (FIC) was evaluated by microdilution chequerboard method with some modifications. The key novelty of the proposed chequerboard technique is the simultaneous deter- mination of the MIC and FIC values (Fig. 1). Assays were performed on 96-well polypropylene microtiter plates on the basis of EOs MIC0 values previously obtained. Six concentrations of Ov and Lv EOs were prepared (8 MIC0, 4 MIC0, 2 MIC0, MIC0, MIC0/2 and MIC0/4). 190 µl of each Ov EO dilution was added on the x-axis across the chequerboard plate, while Ls EO dilutions were dispensed on the y-axis in order to obtain the FIC final concentrations (4 MIC0, 2 MIC0, MIC0, MIC0/2, MIC0/4 and MIC0/8, for each EO) as illustrated in Fig. 1.

Ten µl of bacterial suspension standardized at 0.5 McFarland

standard turbidity were added in each well, except the nega- tive control, which was added with 10 µl of sterile BHI broth. Microplates were incubated at 37 ◦C for 24 h in a humid chamber. FIC determinations were performed in triplicate.

For each replicate, FICI values were calculated using the follow- ing formula:

FICI = FICOv + FICLs

whereFICOv = MICOv in combination/MICOv alone andFICLs = MICLs in combination/MICLs alone.

Authors proposed here for the first time a novel FICI interpre- tation model, where a Synergistic effect (SynA) is detected when FICI value < 1; a Commutative effect (ComA) when FICI value = 1; an Indifferent effect (IndA) when 1 < FICI value ≤ 2 and an Antagonistic effect (AntA) when FICI value > 2.

FICI values were also interpreted following the conventional model suggested by Odds (2003) and EUCAST (2000).

According to Odds (2003) a Synergistic effect (SynO) is observed when FICI value ≤ 0.5; an Indifferent effect (IndO) when 0.5 < FICI value ≤ 4 and an Antagonistic effect (AntO) when FICI value > 4.

According to EUCAST (2000) a Synergistic effect (SynE) is observed when FICI value ≤ 0.5; an Additive effect (AddE) when

0.5 < FICI value ≤ 1; an Indifferent effect (IndE) when 1 < FICI

value < 2 and an Antagonistic effect (AntE) when FICI value 2.

These three different models are summarised in Fig. 2, as general FICI graphical interpretations and as practical applications on the proposed FIC chequerboard.

3. Results and discussion

3.1. Chemical composition of oregano and manuka essential oil

The percentage of the identified compounds was 99.39% for manuka EO and 98.44% for oregano EO (Table 1). Ov EO showed high percentage of Carvacrol (65.93%), followed by p-Cymene (9.33%) and γ-Terpinene (5.25%); thus monoterpene hydrocarbons and oxygenated monoterpenes were the predominant class of com- pounds (22.49% and 71.24%, respectively).

On the contrary Ls EO exhibited high concentration of sesquiter- pene hydrocarbons (55.12%), typically accounting over half of the total oil, with cis-Calamenene as main constituent (15.93%). How- ever, this manuka oil was rich in non terpene compounds (38.83%) and Leptospermone was the compound with the highest per- centage (31.65%), followed by cis-Calamenene (15.93%), Flavesone (6.92%) and iso-Leptospermone (0.11%).

3.2. MIC, FIC and FICI of EOs

For Ov EO, 13 strains showed the same MIC value for all repli- cates. In particular, 8 strains (A, B, C, E, G, H, K and M) showed MIC values of 1:4096 v/v (i.e. 0.240 mg/ml) and 5 strains (D, I, J, L and N) showed MIC values of 1:2048 v/v (i.e. 0.480 mg/ml). In one repli- cate, strain F presented instead a different MIC value (two times 1:4096 v/v and one time 1:2048 v/v).

Ls EO MIC values were 1:2048 v/v (i.e. 0.513 mg/ml) for all strains excepted strain D, for which only one replicate gave a MIC value of 1:4096 v/v (i.e. 0.257 mg/ml).

Both tested EOs showed a remarkable antimicrobial activity against S. aureus via MIC quantification. This effect is well docu- mented for Ov EO (Dal Pozzo et al., 2011; Elgayyar et al., 2001; Fratini et al., 2014; S¸ ahin et al., 2004). More generally, Lamiaceae family is known for its antimicrobial effectiveness due to the high content of several phenol compounds, such as Carvacrol and Thy- mol. It has been demonstrated that the main action mechanism of Carvacrol on bacterial cell consists in a collapse of the proton- motive force and depletion of the ATP pool, with consequent cell death (Thormar, 2010).

Few investigations were carried out on the antibacterial activ- ity of Ls EO (Harkenthal et al., 1999; Lis-Balchin et al., 2000; Takarada et al., 2004). In the last years, researches focused their work mostly on Ls honey and its effect against several microorgan- isms (Henriques et al., 2010; Kumar et al., 2014; Sherlock et al., 2010; Visavadia et al., 2008). Our results highlight a remarkable effectiveness of Ls EO against S. aureus, that may be attributable to the major detected compounds, especially to the presence of β-triketones as Leptospermone. Porter and Wilkins (1999) reported a positive correlation between Ls EO antibacterial activity (mainly against Gram positive bacteria) and its content in β-triketones, such as Leptospermone. Some authors suggest that the activity of β- triketones is correlated to their chemical structure similar to the tetracyclines. However, their modes of action are not yet known (Christoph et al., 2001, 2000).

FIC values for each EO ranged from 0.125 to 1. Fig. 3 shows the

frequencies of the FIC values combinations, expressed as number

of times that the specific combination of EOs dilutions led to a microbial inhibition on the total number of FIC combinations. Four combinations of the EOs dilutions (i.e. FIC Ls of 1.000 with FIC Ov of 0.250 and 0.125; FIC Ov of 0.500 with FIC Ls of 0.500 and 0.250) represent over the 70% of the inhibitory activities.

Two hundred twenty-two FICI values were obtained (as total of the replicates) and they ranged from 0.375 to 1.5. FICI values 0.375 and 0.5 were found respectively two times for strain D. The most frequent FICI value was 1.125, followed by 0.750, 1.250 and 1.000. All the strains showed, at least one time, FICI values of 0.750, 1.000,

1.125 and 1.250. FICI value of 0.625 was recorded in 11 strains (A, B, C, D, E, G, I, J, K, L and N) as well. The highest FICI value (1.500) was detected in 5 strains (A, B, E, H and M).

To the best of our knowledge, this is the first report on the antibacterial activity of Ls EO (mixed) in combination with Ov EO. For their inhibitory activity these EO mixtures could be selected in order to evaluate a potential practical employment.

3.3. FICI interpretations

As reported in Table 2, percentages of FICI classes proposed in this study (SynA, ComA, IndA and AntA) ranged from 0.45 to 5.41%. IndA effect represents the 50.00% of the FICI frequency, followed by SynA and ComA with 34.68% and 15.32%, respectively.

According to Odds (2003) interpretation a SynO of 1.80% and a IndO of 98.20% were detected. Following the interpretation of EUCAST (2000), the same synergistic effect (SynE) percentage could be obtained, while different percentages in AddE (48.20%) and IndE (50.00%) were observed.

Different interpretations were all in accordance on the absence of antagonist effect (AntO, AntE and AntA).

The interpretations of Odds (2003) and EUCAST (2000) agreed in detecting a synergistic effect when FICI is below or equal to 0.500. This decision is strictly linked to the uncertainty of the MIC dilu- tion determination, as reported by Odds (2003). Indeed, the author stated that considering that the variation in a single result places a MIC value in a three-dilution range (mode 1 dilution), there are considerable possibilities for reproducibility errors in the MIC che- querboard. The interpretation proposed in the present work allows to avoid these errors, determining the MIC and the FIC simultane- ously, using the same microarray plate, and the same dilutions (of drugs and microorganisms). In the eventuality of a

variation of the MIC value among the replicates, also the sensibility at the combina- tions of drugs used in the FIC determination is affected in the same way.

As support of this statement, when in the chequerboard method

a MIC value is not in compliance with MIC0 occurred (lower/higher dilution), also the FIC values were influenced in the same way, i.e. MIC values in combination of the FICs are affected by the same dilution difference between MIC and MIC0; when FICIs were cal- culated, the ratio MIC in combination/MIC alone was maintained comparable within the replicates.

The FICI model interpretation proposed in this work, set the syn- ergistic effect when the FICI is below to 1. This decision is related to the assertion that if the same drug was used (drug α) instead two different drugs (drug α and drug β) the combination of the dilutions MICα/2 and MICα/2 (both with FICα value = 0.5, FICI = 1) or any other combination which leads to FICI = 1 (in the case of not two fold dilutions, as MICα/4 and 3MICα/4 etc.) is equal to use the MICα dilution. In that case, an observed inhibitory effect has to be associate with the use of the threshold dose (MICα); if again we take into consideration two drugs, the part of drug α replaced with the part of drug β that produced an inhibitory effect with FICI equal to 1 is to be considered as a complementary part that does not change the effect neither negatively nor positively (Commutative effect, ComA). When the dose of one drug is MICα/2 and the other drug is at a dilution under the MIC/2 (MICβ in combination < MICβ/2) the doses of the drugs are less than the quantity needed if one drug is used (MICα/2 + < MICα/2). In that case, the total dose of drug is reduced and a synergistic effect (SynA) would lead to the use of a lower quantity of α and β compared to the single drugs effective dose.

When 1 < FICI 2, one drug presented a FIC value equal to 1, this means that the dose of α in the combination is equal to the MICα alone dilution; any FIC value of β under 1 (dose in combina- tion < MICβ alone dilution) do not change the effect of the inhibition due to the dose of α. At least when FICβ=1, both the drugs are in the concentration equal to their MIC values, the indifferent effect (IndA) do not modified the effectiveness of α and β.

When FICI > 2 at least one drug in combination is at a dilution higher than its effective dose (MIC) and the other drug is at a dilu- tion higher or equal to the MIC dose (FICα > 1; FICβ 1); in this case the inhibition effects expected by both the concentrations of α and β are modified mutually; an antagonist effect occurred (AntA).

4. Conclusions

In our proposed interpretation, the thresholds for synergy and antagonism are in accordance with Odds' (2003) interpretation (FICI 0.5 and FICI > 4). However, using the proposed MIC/FIC che- querboard the MIC quantification error could be overcome (synergy when FICI < 1; antagonism when FICI > 2). The commutative effect (FICI = 1) was included in Odds (2003) and EUCAST (2000) inter- pretations (considering the MIC error) respectively as indifferent or additive effect.

The proposed MIC/FIC chequerboard and the following FICI interpretation, showed that Ov and Ls EOs could be a practically used in combination against S. aureus for their synergistic activity. Synergy of natural products should be further investigated in order to have valid alternative antibacterial tools against pathogen and spoilage microorganisms.

Presence of multidrug resistant microorganisms represent an increasingly widespread problem. EOs may be a valid alternative to chemotherapic drugs and their use is growing in human/veterinary medicine, as well

as in food industries. Synergisms of EOs appear to have a perspective employment for reduce the effective dose and at the same time the collateral effects due to their different mechanisms of action.

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Table 1
Chemical composition of oregano (*Ov*) and manuka (*Ls*) essential oils.

Compounds	LRI[a]	*Ov*	*Ls*
Tricyclene	933	–	1.02
Myrcene	991	2.2	0.14
α-Terpinene	1018	2.05	–
p-Cymene	1026	9.33	–
γ-Terpinene	1062	5.25	–
trans-Sabinene hydrate	1098	1.78	–
Carvacrol	1299	65.94	–
α-Cubebene	1351	–	3.26
α-Copaene	1376	–	4.27
β-Caryophyllene	1418	3.72	2.1
α-Guaiene	1439	–	1.81
cis-Muurola-3.5-diene	1448	–	5.45
trans-Cadina-1.6.4-diene	1470	–	3.56
γ-Muurolene	1477	–	1.37
β-Selinene	1483	–	3.3
trans-Muurola-4-(14).5-diene	1492	–	1.02
Valencene	1493	–	3.95
cis-Calamenene	1521	–	15.93
trans-Cadina-1 (2).4-diene	1533	–	4.25
Flavesone	1547	–	6.92
Globulol	1583	–	1.15
Leptospermone	1631	–	31.65
Cubenol	1641	–	1.22
Monoterpene hydrocarbon		22.49	1.16
Oxygenated monoterpenes		71.24	0.28
Sesquiterpenes hydrocarbon		4.2	55.12
Oxygenated sesquiterpenes		0.4	4
Non terpenes		0.05	38.83
Phenyilpropanoids		0.06	–
Total		98.44	99.39

Other compounds were detected but not summarised because <1%. The minority compounds detected were for *Ov*: α-Thujene, α-Pinene, α-Fenchene, β-Pinene, 3-Octanol, α-Phellandrene, Limonene, (*E*)-β-Ocimene, *cis*-Sabinene hydrate, Terpinolene, Camphor, Borneol, 4-Terpineol, α-Terpineol, Isobornyl acetate, Thymol, Eugenol, α-Humulene, β-Bisabolene and Caryophyllene oxide; for *Ls*: 3-methyl-3-butenyl-3-methyl butanoate, α-Ylangene, β-Elemene, Isovaleric acid benzyl ester, α-Gurjunene, β-Copaene, *allo*-Aromadendrene, α-Muurolene, Germacrene A, δ-Amorphene, (*E.E*)-α-Farnesene, *trans*-γ-Cadinene, α-Calacorene, Spathulenol, Guaiol, 5-*epi*-7-*epi*-α-Eudesmol, iso-Leptospermone, Selin-11-en-4-α-ol (kongol) and Occidentalol acetate; for both the EOs: 1.8-Cineole. All the mentioned compounds were utilised for calculating the total percentage of each class of constituents.

[a] Linear Retention Index with respect to a series of normal alkanes on a HP-5 MS column.

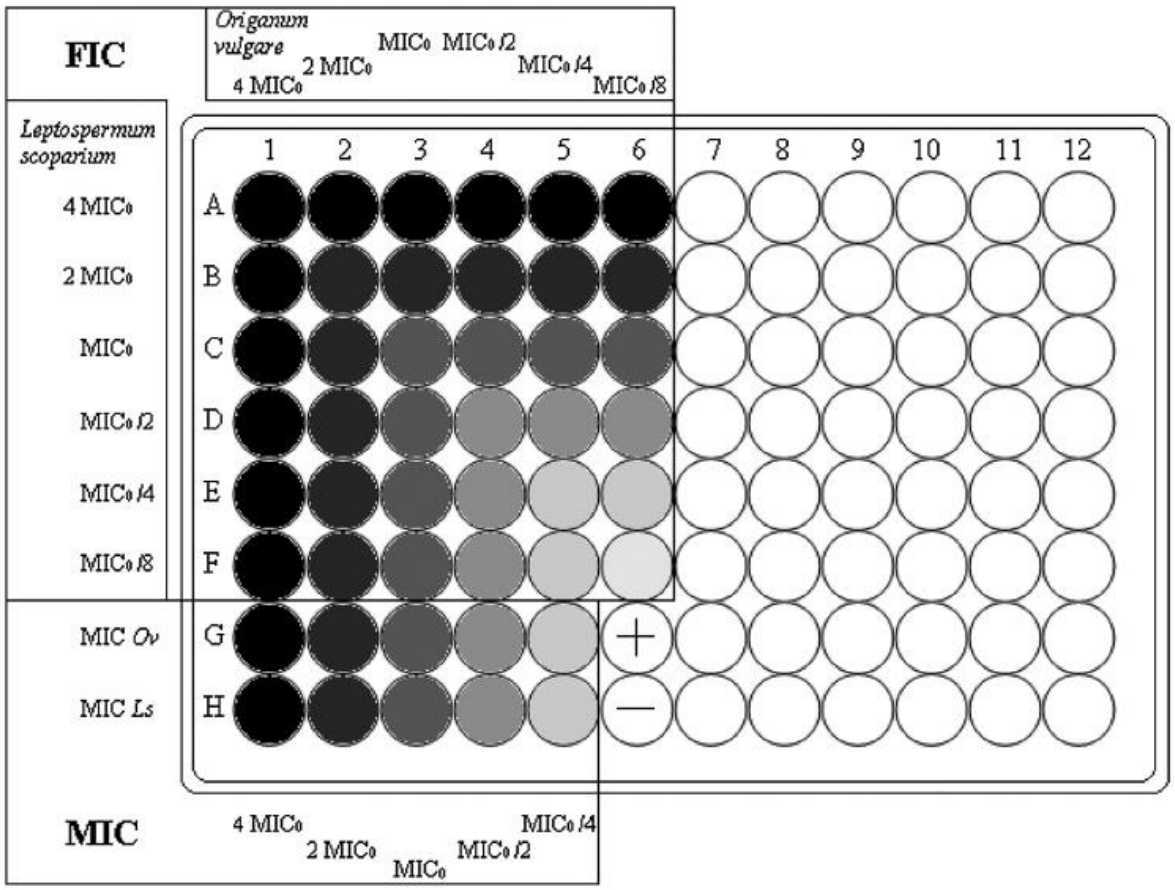


Fig. 1. Microdilution chequerboard method for simultaneous MIC and FIC determination.

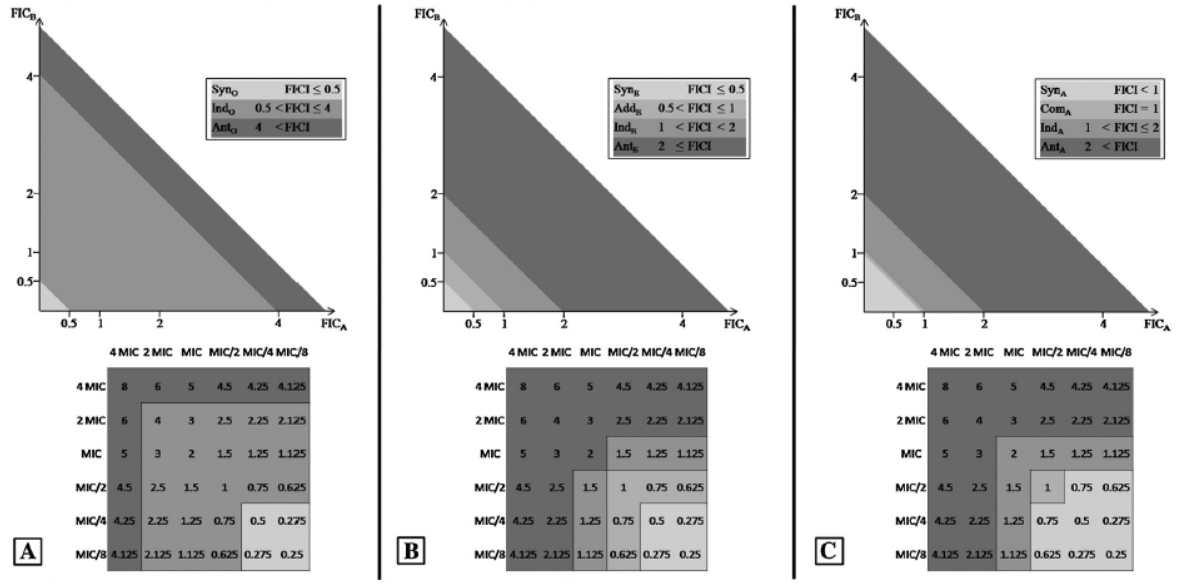
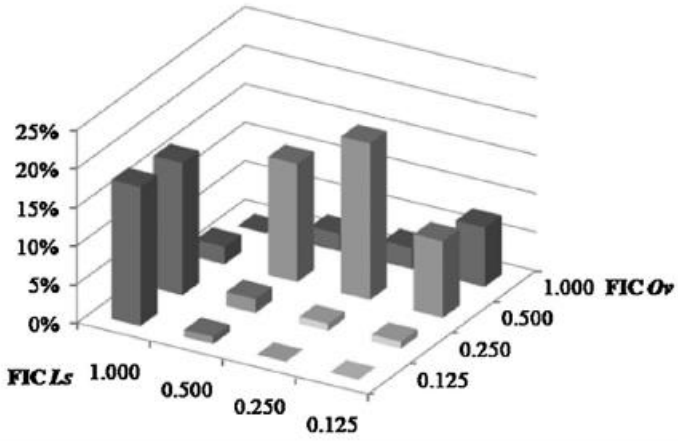


Fig. 2. General FICI graphical interpretations and practical applications on the proposed MIC/FIC chequerboard.

Table 2
FICI interpretation percentages.

	Strains														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Total
Syn_O %				1.80											1.80
Ind_O %	7.21	7.21	6.76	2.70	7.21	8.11	7.21	8.11	7.21	7.21	7.21	6.76	8.11	7.21	98.20
Ant_O %															0.00
Syn_E %				1.80											1.80
Add_E %	2.70	2.70	4.05	1.80	2.70	4.05	4.05	2.70	3.60	4.05	4.05	4.95	2.70	4.05	48.20
Ind_E %	4.50	4.50	2.70	0.90	4.50	4.05	3.15	5.41	3.60	3.15	3.15	1.80	5.41	3.15	50.00
Ant_E %															0.00
Syn_A %	1.80	1.80	2.70	3.15	1.80	2.70	2.70	1.80	1.80	2.70	2.70	4.50	1.80	2.70	34.68
Com_A %	0.90	0.90	1.35	0.45	0.90	1.35	1.35	0.90	1.80	1.35	1.35	0.45	0.90	1.35	15.32
Ind_A %	4.50	4.50	2.70	0.90	4.50	4.05	3.15	5.41	3.60	3.15	3.15	1.80	5.41	3.15	50.00
Ant_A %															0.00

FICI interpretations as suggested by Odds (2003): Synergistic effect (Syn_O), Indifferent effect (Ind_O) and Antagonist effect (Ant_O); by EUCAST (2000): Synergistic effect (Syn_E), Additive effect (Add_E), Indifferent effect (Ind_E) and Antagonist effect (Ant_E); by Authors: Synergistic effect (Syn_A), Commutative effect (Com_A), Indifferent effect (Ind_A) and Antagonist effect (Ant_A).



	FIC values of *Ls*			
FIC values of *Ov*	**1.000**	**0.500**	**0.250**	**0.125**
1.000	-	**2.25**	**2.70**	**7.66**
0.500	**2.25**	**15.32**	**20.27**	**9.91**
0.250	**17.12**	**1.80**	**0.90**	**0.90**
0.125	**18.02**	**0.90**	-	-

Fig. 3. Frequencies (%) of FIC values for oregano (*Ov*) and manuka (*Ls*) essential oils against *Staphylococcus aureus* wild strains.