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Interaction between terpenes and penicillin on bacterial strains resistant to *beta*-lactam antibiotics

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INTRODUCTION

A variety of medicinal plants possess the capacity to synthesize aromatic compounds called essential oils, which are obtained either by hydrodistillation or by expression. These oils are complex mixtures of organic compounds, including monoterpenes and sesquiterpenes. Beneficial properties for human and animal health have been attributed to these compounds such as: antitumoral activity, antioxidant efficiency and antimicrobial activity (Zygadlo and Juliani, 2001). The antimicrobial activity of some essential oils from some native species of Argentina was tested. Good antimicrobial activity was observed in Gram positive bacteria while reduced inhibitory activity was observed in Gram-negative bacteria, particularly on *Pseudomona aeruginosa*.

The indiscriminate use of antibiotics gave rise to the appearance of multiresistant strains, *e.g.*, methicillin resistant *Staphylococcus aureus* (MRSA), which at the present time is one of the main causal agents of intrahospitalarial infections in the world. There are also strains naturally resistant to these substances, such as Gram-negative bacteria.

In this work we want to study the influence of a terpenic compound on the mode of action of an antibiotic of conventional use, *e.g.*, penicillin, which is ineffective against MRSA, and is not specific for Gram-negative strains, such as *Escherichia coli*. The combination of terpenes and antibiotics could result in a sinergistic effect in order to eliminate this microorganism and/or other natural resistant ones, such as Gram-negative bacteria, in particular *P. aeruginosa*.

Aim: The aim of this work was to study the antimicrobial activity of terpenes, and to detect sinergistic activity between terpenes and antibiotics, *e.g.*, penicillin.

METHODOLOGY

l. Microbial strains. *Staphylococcus aureus* MRSA ATCC 25923, *Escherichia coli*.

2. Terpenes.

Carvacrol, citronellol, eugenol, geraniol, menthol, menthone, myrcene, thymol.

3. Determination of the Minimum Inhibitory Concentration of terpenes and penicillin.

It was performed by the broth microdilution technique, according to Mann and Markham (1998).

4. Detection of associations between terpenes and antibiotics by the modified technique of Didry.

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The methodology was a combination of the broth microdilution technique (Mark and Markham, 1998) with the technique described by Didry *et al*. (1993) in which the Fractional Inhibitory Concentration (FIC) is determined.

RESULTS AND DISCUSSION

l. MIC of terpenes and penicillin.

The following terpenes showed activity against MRSA strain: eugenol, geraniol, thymol and carvacrol, standing out the two latter with the less MIC values of 30.15 mg/ml and 15.25 mg/ml, respectively. Concerning the antimicrobial activity of terpenes against *E. coli*, its worth to be mentioned the inhibitory activities of carvacrol with a MIC of 7.62 mg/ml, and thymol with a MIC of 15.07 mg/ml. This bacteria was also inhibited by eugenol and geraniol showing MIC values of 66.87 mg/ml and 222.25 mg/ml, respectively. The rest of the terpenes did not inhibit growth of any of these microorganisms.

The antimicrobial activity of penicillin against MRSA was 120,000 UI/ml, which is an expected result since MRSA strains have a diminished affinity for *beta*-lactamic antibiotics (Chambers, 1997). The MIC of this antibiotic with *E. coli* was 60,000 UI/ml, demonstrating that a high concentration of this antibiotic was required to inhibit the growth of this microorganism. This fact can be explained because penicillin is not specific for Gram-negative bacteria, which possess high resistance to antibiotics, possibly due to the presence of the external membrane (Helander *et al*., 1998).

Table 1: Minimum Inhibitory Concentration of terpenes (mg/ml) and penicillin (UI/ml)

Terpenes	MRSA	*E. coli*
Carvacrol	15.25	7.62
Carvone	NI	NI
Citronellol	NI	NI
Eugenol	133.75	66.82
Geraniol	55	222.25
Menthol	NI	NI
Menthone	NI	NI
Myrcene	NI	NI
Thymol	30.15	15.07
Penicillin	120,000	60,000

Ref: NI: No inhibition

2. Associations between terpenes and penicillin against methicillin resistant *Staphylococcus aureus*.

High antibiotic concentrations are required in order to inhibit MRSA strain development, thereby new alternatives are being studied for the treatment of these microorganisms. The combination of terpenes and antibiotics could produce a sinergistic effect, eliminating this microorganism. Different combinations of terpenes and penicillin were carried out in order to evaluate the effect of the combined action of these compounds on the studied strains.

The results obtained with the combinations of terpenes showed that carvone/penicillin was totally sinergistic, while thymol/penicillin was antagonistic with *S. aureus* MRSA. The associations of citronellol, eugenol, geraniol, menthol, menthone and myrcene, each one combined with penicillin showed to be indiferent, independently of each antimicrobial activity when they were used alone (Table 2).

Table 2. Fractional Inhibitory Concentration of terpenes with penicillin on MRSA

Terp./Penicillin	FIC	Association Type
Carvone	0.0078	Total sinergism
Citronellol	1	Indiference
Eugenol	1.0078	Indifference
Geraniol	1.0078	Indifference
Menthol	1	Indiference
Menthone	1	Indiference
Myrcene	1	Indiference
Thymol	3,98	Antagonism

3. Associations between terpenes and penicillin against *Escherichia coli*.

Gram-negative bacteria are more resistant to antibiotics than the Gram-positive bacteria. Constantly new therapeutic alternatives are being looked for in order to stop the development of these microorganisms. The use of terpenes combined with drugs could enhance the income of the last ones to the cell. Antibiotics could allow a best transport of the latter until reaching its bacterial cell target owing to the lipidic nature of the terpene. (Harris, R. 2003)

Associations between thymol/penicillin and eugenol/penicillin showed excellent sinergistic activities with FIC values of 0.15 and 0.168, respectively, while the association

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myrcene/penicillin was antagonistic. The other associations were indifferent (Table 3).

Table 3. Fractional Inhibitory Concentration of terpenes with penicillin against *E. coli.*

Terp./Penicillin	FIC	Association Type
Carvacrol	2	Indifference
Citronellol	1.03	Indifference
Eugenol	0.16	Total sinergism
Geraniol	1.5	Indifference
Menthol	1.5	Indifference
Myrcene	10	Antagonism
Thymol	0.15	Total sinergism

CONCLUSIONS

* Association between carvone and penicillin was effective on MRSA.

* Associations between eugenol/penicillin and thymol/penicillin were effective against Gram negative strains, such as *E. coli*, resistant to some *beta*-lactamic antibiotics.

* Combinations between terpenes and antibiotics could be considered promising chemotherapics for treatment of diseases produced by these microorganisms.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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