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Antistaphylococcal synergistic interaction between the essential oil of *Rosmarinus officinalis* L. and ciprofloxacin

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ABSTRACT

This work is, in addition to the phytochemical study, to evaluate the anti-staphylococcal activity of the essential oil of Rosmarinus officinalis L. and detect some kind of synergy interactions with ciprofloxacin. Essential oil is extracted by steam distillation in a Clevenger type apparatus (Clevenger, 1928), analyzed by gas chromatographic coupled with mass spectrometry. Aromatogram , minimum inhibitory concentrations (MIC) and bactericidal (MBC) allowing to evaluate the anti-Staphylococcus aureus activity of essential oil alone or in combination with ciprofloxacin were studied . The report MBC/MIC of the essential oil of rosemary is 1, which allows the oil to qualify as a bactericide. Ciprofloxacin is bactericidal with a report MBC/MIC equal to 2, which confirms the summary of the characteristics of this product. The MICs of the two products are used to calculate the fractional inhibitory concentration (FIC), it is less than 1, which is used to identify a synergistic interaction between ciprofloxacin and the essential oil of rosemary . Interaction between the essential oil of Rosmarinus officinalis L. and ciprofloxacin is synergistic. It may enhance the anti-Staphylococcal activity and reduce the number and severity of side effects of this fluoroquinolone; especially those that are dose-dependent.

Keywords: Antibacterial activity, synergy, *Staphylococcus aureus*, essential oil, *Rosmarinus officinalis* L., ciprofloxacin.

INTRODUCTION

National Agency for Medicines and Health Products Safety (ANSM) defines essential oils as follows: "Fragrant product, usually complex composition obtained from a vegetable raw material botanically defined, by steam drive water, by dry distillation or by a suitable mechanical process without heating. The essential oil is usually separated from the aqueous phase by a physical method involving no significant change in its composition "[1].

Essential oils are the means of plant natural defense against microorganisms. These properties are evaluated in vitro and rarely in vivo in humans as in animals, making this class a real mine of evaluation and exploration in the field of scientific research [2].

Abuse of antibiotics use, ill-treatment adherence, interindividual variabilities kind pharmacogenetics and unjustified indications antibiotics are the trigger factors of antibiotic resistance [3-7], hence the need to search for another alternative treatment.

This work is, in addition to the phytochemical study, to evaluate the anti-staphylococcal activity of the essential oil of *Rosmarinus officinalis* L. and detect some kind of synergy interactions with ciprofloxacin.

EXPERIMENTAL SECTION

Plant material: Sample was collected in March in the province Chtouka Ait Baha, Sous-Massa-Draa (Southern Morocco) area. The part used is the flowering tops.

Studied microorganism: *Staphylococcus aureus* was chosen due to its frequency and its pathogenicity. The bacterial strain is a lot of ATCC (American Type Culture Collection 25923). It is maintained by subculture on nutrient agar favorable to its growth for 24 h in the dark at 37 ° C [8].

Antibiotic: It is a synthetic antibiotic which is ciprofloxacin; it is a second generation quinolone or fluoroquinolone [8]. It is active on *Staphylococcus* at higher doses or concomitantly administration with other appropriate antibacterial agents [9].

Extraction of essential oils [10]: The extraction of essential oils was made by hydrodistillation in a Clevenger-type apparatus (Clevenger, 1928) for 1:30, 200 g of fresh plant material with 1 liter of water in a 2 l flask. This technique was developed by adding cooling device (ice) to prevent the return and the loss of the volatile product.

The essential oil yield was determined relative to the dry matter. The essential oil was stored at 4 ° C in the dark in the presence of anhydrous sodium sulfate. Prior to analysis GC (gas chromatography) and GC / MS (gas chromatography coupled to mass spectrometry) the essential oil was diluted in methanol (1%, v / v).

Chromatographic analysis: Qualitative and quantitative analysis of components of essential oils was determined by gas chromatographic coupled to mass spectrometry.

The sample changer is automatic, the volume injected is 1 µl, the carrier gas is helium, column used is type Elite-5ms whose length is 30 m and a diameter of 0.25 µm.

The injection temperature is 250 ° C and that of the transfer line is 200 ° C, the ionization potential of 70eV, the source temperature is 200 ° C. The characteristics of each device are shown in table 1.

Table-1 Characteristics of different devices chromatograph

Device	Characteristics
Column	Capillairehp-5 column (5% phenyl methyl siloxane) (30 m x 0.25 mm, film thickness 0.25 µm)
Detector	FID (temperature: 250 ° C) supplied with a gas mixture of H2 / air and a gas injector Split-splitless (temperature: 250 ° C)
Injection volume	1 µl
Injection mode	Split (leakage Report: 1/50, flow: 66 ml / min)
Vector gaz	Nitrogen at a flow rate of 1.7 ml / min
Column temperature	Programmed from 50 to 200 ° C at a rise of 4 ° C / min and an isotherm for 5 min at the final temperature
Computer system	« HP ChemStation »
Mass spectrometer	HP 5973 Series
Fragmentation	Electron impact in a field of 70 eV

Microbiological process:

Aromatogram: It has the same principle as antibiogram, it can determine the zone of inhibition caused by the action of essential oils to microorganisms and thus evaluate the inhibitory activity of these products [11].

The petri dishes were flooded with 100 µl of a suspension of 10^8 bacteria / ml of *Staphylococcus aureus* prepared with sterile water and dried under a laminar flow hood. A dilution series was performed using a 0.2% agar solution in order to have concentration range of about 5, 10, 15, 50 and 100%.

Sterile filter paper disks of 6 mm in diameter, impregnated with essential oil are then deposited on the agar. The incubation is done in the oven for 24 h at 37 ° C.

The interpretation of results is made according to the diameter of inhibition [12]:
• Resistant bacterium (D <6 mm)
• Intermediate Bacteria (D 13 mm 6 mm)
• Sensitive bacteria (D> 13 mm)

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The MIC is the lowest antibiotic concentration that inhibits all visible growth of bacteria after 18 to 24 h incubation respecting the characteristics of each seed; it allows evaluating the bacteriostatic effect of an antibacterial product.

MBC is the lowest concentration of an antibacterial killing product over 99.99% germ after incubation at 37 ° C for 18 to 24 h, it characterizes the bactericidal effect of an antibacterial product.

The MIC and MBC are determined according to the liquid medium dilution macromethod described by El Amri et al [13], to promote contact essential oil / germ, agar solution at 0.2%.was used.

RESULTS AND DISCUSSION

Chromatographic analysis of the essential oil of rosemary showed that the major components are 1,8-cineol, respectively (41.04%), camphor (14.35%), alpha-pinene (12.89 %), beta-pinene (8.36%) and camphene (4.32%) (Table 2).

Table-2 Chemical composition of the essential oil of rosemary

N°	Constituent	Percentage (%)
1	1,8-cineol	41.04
2	Camphre	14.35
3	α-pinene	12.89
4	β-pinene	8.36
5	Camphene	4.32
6	Limonene	2.92
7	β-trans-terpineol	2.13
8	E-caryophyllene	1.95
9	Myrcene	1.73
10	α-terpinene	1.53
11	α-terpineol	1.27
12	Terpin-4-ol	0.92
13	α-terpinen-7-al	0.91
14	Linalool	0.82
15	E-β-ocymene	0.69
16	α-humulene	0.46
17	Oxyde de caryophyllene	0.43
18	1,4-cineol	0.41
19	Vérbenone	0.39
20	Terpinolene	0.36
21	γ-terpinene	0.31
22	3-octanne	0.24
23	α-thujene	0.23
24	α-phellandrene	0.23
25	Tricyclene	0.21
26	δ-cadinene	0.21
27	2-β-ocymene	0.16
28	α-campholenol	0.15
29	Thuja-2,4-(10)-diene	0.12
30	Sabinene	0.11
31	δ-3-carene	0.09
Total		**99.94**

It is 1,8-cineol chemotype, these results are consistent with those of Khia et al. and respect the AFNOR NF ISO 4730 [14].

Khia et al. also showed a difference in the chemical composition of the essential oil of rosemary and depending on the source region [14].

The essential oil yield was 2.93%, it is higher than that found by Khia et al. and Fechtal et al. [14]. This powerful performance may be due to the biological quality of the crop and the cooling devices that mounted on the Clevenger apparatus.

Aromatogram enabled to deduce that *Staphylococcus aureus* is very sensitive to the essential oil of rosemary (Tables 3 and 4), this activity may be due to the high content of 1,8-cineol and camphor, the latter is described in literature as a potent antibacterial agent [15].

Table-3 Diameters inhibition of *Rosmarinus officinalis* L. essential oil

Essential oil concentration (v / v)	5%	10%	15%	50 %	100 %
Inhibition diameter in mm	21 +/- 1,6	23 +/- 1,9	25 +/- 2,1	26 +/- 2,4	28 +/- 2,6

Table-4 MIC and MBC of *Rosmarinus officinalis* L. essential oil

essential oil	MIC in mg/ml	MBC in mg/ml
Rosmarinus officinalis L.	0,5	0,5

The inhibition diameter of ciprofloxacin is lower than the rosemary E.O (Table 5), however an association between the two allows the potentiation of the effect of ciprofloxacin (Table 6).

The report MBC/MIC of the essential oil of rosemary is 1, which allows the oil to qualify as a bactericide.

Table-5 diameters of inhibition, MIC and MBC of ciprofloxacin

Antibiotic	Inhibition diameter in mm	MIC in mg / ml	MBC in mg / ml
Ciprofloxacin (5µg)	22+/- 1,2	$0,5\ 10^{-3}$	$1\ 10^{-3}$

Table-6 Inhibition diameter of the combination of *Rosmarinus officinalis* L. essential oil and ciprofloxacin

Association	Inhibition diameter in mm
E.O + Ciprofloxacin	26+/-1,7

Ciprofloxacin is bactericidal with a report MBC / MIC equal to 2, which confirms the summary of product characteristics [15].

The MICs of the two products permit calculating the FIC (Table 7), it is less than 1, which is used to identify a synergistic interaction between ciprofloxacin and the essential oil of rosemary.

The synergy between essential oils and certain antimicrobial drugs has been studied by some researchers [16].

Table-7 Fractional Inhibitory Concentrations (FIC)

FIC *ciprofloxacin*	FIC rosemary E.O	FIC index	Interaction type
0.400	0.041	0.441	synergie

Giordani and Kaloustianont [17], have shown that concomitant use of essential oil of *Cinnamomum cassia* and *Thymus vulgaris* thymol chemotype with amphotericin B has significantly decreased the MIC 80 % of the latter, this explains an increase antifungal power of amphotéricin B.

Mechanism of action of essential oils is poorly understood, they do not act the same intensity on microorganisms, which suggests a selectivity of action. This selectivity may be due to various components that work together [18].

Rhayour et al. talk about a cell lysis mechanism following an action on the membrane in the evaluation of antibacterial activity (gram + and gram -) of essential oils of cloves and oregano simultaneously with thymol and eugenol [19].

The synthesis of the results of different studies shows that the mechanisms of essential oils are [11]:

• Alteration of the cell wall;
• Alteration of membrane proteins;
• Degradation of the cytoplasmic membrane;
• Leakage of the cell contents;
• Cytoplasmic coagulation;
• Exhaustion of the force of movement of protons.

Bactericidal activity of ciprofloxacin results from the inhibition of topoisomerase II (DNA gyrase) and topoisomerase IV, required for replication, transcription, repair and recombination of Bacterial DNA (deoxyribonucleic acid) [9].

The association of a bactericidal and bacteriostatic antibiotic induced antagonism [20], an association between two bactericides causes synergy, however, the interactions between antibiotics remain complex [21].

Based on these data, the mechanism of synergistic interaction between the essential oil of rosemary and ciprofloxacin is that essential oil ease penetration of ciprofloxacin within the bacteria by altering the cytoplasmic membrane and its components and by other actions of the essential oils described above on the different parts of the bacteria. These results are promising new research for a fixed association between essential oils and antibiotics.

CONCLUSION

Essential oils occupy an increasingly important place in medicine and cosmetology. This study evaluated the anti-staphylococcal activity of the essential oil of rosemary and identified synergistic interaction between the oil and ciprofloxacin. These results suggest new studies on multi-resistant clinical strains. The association with ciprofloxacin allows to increase the anti-staphylococcal activity and reduce the number and severity of side effects of this fluoroquinolone; especially those that are dose-dependent. We must be vigilant about side effects and toxicity of essential oils in a misuse or abuse, which requires the advice of a healthcare professional.

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