



Antibacterial effect of synergy of two essential oils extracted from marjoram (*origanum majorana*) in the region of salé and oregano (*origanum vulgare*) in the region of ouazzane, Morocco

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ABSTRACT

The objective of this work is to evaluate the antimicrobial activity of the combination of two essential oils extracted from *Origanum majorana* and *origanum vulgare*.

Each one of this two essential oils has a significant antibacterial activity against resistant microorganisms. In the context of Moroccan flora's valorization, we are interested by species of the Lamiaceae family. It's a family widely used by Moroccan population as a spice. The essential oils of marjoram and oregano are obtained by hydro distillation in a Clevenger type apparatus. The yield of essential oils obtained from the leaves of marjoram and oregano is respectively (2.50% and 2.34%). The inhibitory effect of the synergy of two essential oils was not the same against the tested strains (*Escherichia coli* ATCC 25921, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosae* ATCC 27853, *Acinetobacter sp*, *Staphylococcus aureus* ATCC 25923). The bactericidal activity is remarkable against all tested strains. However, the largest zone of inhibition is 25 mm observed in the case of *Acinetobacter*.

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KEYWORDS

Marjoram;
Oregano;
Essential oils;
Antimicrobial activity;
Hydro-distillation;
Pathogens.

INTRODUCTION

Among the multitude of aromatic plants existing in the world, we are particularly interested in the kind *Origanum*. The Marjoram (*Origanum majorana*) and oregano (*Origanum vulgare*) are plants of the family Lamiaceae, this last includes 187 genera and 3000 species, it is the most homogeneous of the gamopetalous sub class, and most genres are rich in essential oils^[1]. The Marjoram does not

exist in the wild; it is cultivated as plant condiment for its aromatic leaves. It is a species very close to oregano, which has leaves of 1 to 2 cm long, opposite, grayish green, whole oval; its flowers are small, white or purple, arranged in groups tight leaf axils with two spoon-shaped bracts^[2]. It is a very aromatic plant used in cooking, particularly in Mediterranean culinary dishes, its essential oil is known for its antiseptic property^[3].

Oregano is a widespread perennial plant, it is

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one of the rare spices that originally pushed in Morocco. It is known for its antiseptic activity^[4, 5] and its therapeutic activity in traditional medicine^[6]. Marjoram badly supports the low temperature, it is grown as an annual plant. It does not ask maintenance neither enough water and it supports dry lands.

For reasons of the scientific confirmation, we are interested in the antibacterial effect of synergy of essential oils from two plants: marjoram (*Origanum majorana*) in the Region of Salé and oregano (*Origanum vulgare*) in the region of Ouazzane. These essential oils will be tested against some strains collected from pathological samples.

MATERIALS AND METHODS

Materials and methods

Material

Plant material

The plant material marjoram (*Origanum majorana*) was collected in June 2013 from the region of sale and the plant material oregano (*Origanum vulgare*) was collected in July 2012 from the region of Ouazzane. Samples were drying in darken

and well ventilated area at room temperature for 15 days to facilitate storage. The leaves used for the extraction of essential oils were separated from the rest of the plant and kept in clean and airy bags.

Micro-organisms studied

The antibacterial activity was evaluated against different microorganisms showing in TABLE 1.

These bacterial strains were purified by subculture on Mueller-Hinton agar and incubated for 24h in at 37°C; they are the cause of several nosocomials infections (urinary, intestinal, respiratory, etc.)^[7, 8].

Methods

The extraction of essential oils

The extraction of essential oils of marjoram (*Origanum majorana*) was carried out by hydro-distillation in a Clevenger-type apparatus^[9]. Today the hydro-distillation remains the most sought method in the industry for extraction of essential oils. The estimation of the yield of essential oil is based on the weight of total dray matter (vegetative). The obtained essential oils are collected and stored in a refrigerator at 4°C in dark bottles to protect them from efficacy of the heat and light in the presence of an-

TABLE 1 : Tested micro-organisms

Microbial group	Tested strains	Origin of strain
Gram negative	EscherichiacoliATCC25921	UTI
	Klebsiella pneumoniae	UTI
	Enterobacter cloacae	UTI
	PseudomonasaeruginosaeATCC27853	Skin infection
Gram positive	Acinetobacter sp	UTI
	Staphylococcus aureusATCC25923	UTI

*UTI: Urinary tract infection

TABLE 2 : Methods used in the study of antimicrobial potency

Methods	Used for	Description	Incubation	Play
Technical of the aromatogram	Bacteria	Filing of filter paper discs of 6mm diameter before and oil-impregnated essential to surface of the medium(Mueller Hinton Agar), planted in boxes flood ^[12]	37 °C/ 24	Measurement diameter inhibition.
Method contact direct	Bacteria	From a stock solution and various dilutions prepared, all set to contact with the medium. The bacteria were sown are a per spot ^[13]	37°C/24h	presence or absence of bacterial growth
Nature of the antibacterial Activity	Bacteria	A sample of the agar disk the surface of Mueller-Hinton agar is made	37 °C/ 24h	presence or absence of bacterial growth

hydrous sodium sulfate^[10]. The method applied was that of Clevenger which is described in the European Pharmacopoeia and the 9th edition of the French Pharmacopoeia.

The yield of obtained essential oils was calculated by the following formula^[11]:

$$(\text{EOY}) \text{Essential oils yield (\%)} = W_1 / W_2 \times 100$$

W1 = net weight of oils from dried leaves (grams); W2 = total weight of fresh leaves (100grams)

Microbiological procedure

The used methods are summarized in TABLE2:

RESULTS AND DISCUSSION

The essential oil of marjoram obtained is dark to pale yellow color and as wet smell, fine hot and delicate. It has a yield up to 2.50% from the leaves and the essential oil of oregano is obtained yellowish to dark brown color and herbaceous smelling. It has a yield up to 2.34% from the leaves.

The results of antimicrobial activity of synergy of essential oils from two plants: marjoram (*Origanum majorana*) and oregano (*Origanum vulgare*) are showed in TABLE 4.

The experimental results presented in TABLE 4 show that the synergy of essential oils from two plants: marjoram (*Origanum majorana*) in the Region of Salé and oregano (*Origanum vulgare*) in the region of Ouazzane has a very good activity against all bacteria except *Pseudomonas aeruginosae*, the same activity on Gram-positive and Gram-negative

bacteria was noticed. The maximum inhibition of synergy of essential oil was against *Acinetobacter* (25mm), *Staphylococcus aureus* (22mm), followed by the other three strains of *Escherichia coli* ATCC 25921(20mm), *Klebsiella pneumoniae* (20mm) and *Enterobacter cloacae* (18mm). These species are sensitive to the synergy of the two oils, while *Pseudomonas aeruginosae* exhibited a resistance to the synergy of two oils.

According to the inhibition zones generated by the synergy of two oils: marjoram and oregano, we are confirmed the inhibitory potential of this essential oil by determination of the minimum inhibitory concentration (MIC). The results of minimum inhibitory concentration (MIC) of the synergy of two oils are summarized in TABLE 5.

In fact, the synergy of two oils: marjoram and oregano, showed a significant inhibitory effect against microorganisms studied. All microbial strains were inhibited at a concentration of 1/50 (V/V). *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter* are more resistance to this synergy of two oils with an MIC of 1/50(V/V). Where as *Acinetobacter* and *Staphylococcus aureus* have a lowest MIC which was 1/100(V/V). The synergy of two oils is very active on all strains tested except *Pseudomonas* which was more resistant. Following these results, the synergy of two oils has shown very interesting antibacterial characteristics against the micro-organisms tested. The results of minimum bactericidal concentration (MBC) the synergy of two oils: marjoram and oregano are summarized in TABLE 6.

TABLE 3 : The essential oil content of *origanum vulgare* and *origanum majorana*

Plant	marjoram	oregano
Quantity	100 g	100 g
Yield	2, 50%	2,34%

TABLE 4 : Activité antimicrobienne de la synergie des deux huiles essentielles : la marjolaine (*origanum majorana*) et l'origan (*origanum vulgare*)

Bacteria	Diameter of inhibition Zones (mm)
<i>Klebsiella pneumoniae</i>	20 mm
<i>Acinetobacter</i>	25 mm
<i>Staphylococcus</i>	22mm
<i>Pseudomonas</i>	-
<i>Escherichia coli</i>	20 mm
<i>Enterobacter</i>	18mm

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TABLE 5 : The results of minimum inhibitory concentration (MIC) for the synergy of two oils: marjoram and oregano

Bacteria	Concentration(V/V)							Témoïn
	1/10	1/25	1/50	1/100	1/200	1/300	1/500	
Klebsiellapneumoniae	-	-	-	+	+	+	+	+
Acinetobacter	-	-	-	-	+	+	+	+
Staphylococcus	-	-	-	-	+	+	+	+
Escherichia coli	-	-	-	+	+	+	+	+
Enterobacter	-	-	-	+	+	+	+	+

Inhibitrice, + Croissance

TABLE 6 : Results of minimum bactericidal concentration (MBC) for the synergy of two oils: marjoram and oregano

Bactéries	Klebsiella Pneumonia	Acineto-bacter	Staphylo-coccus	Escherichia coli	Enterobacter
(CMB)(V/V)	1/100	1/100	1/25	1/100	1/50

The bactericidal activity appears highly variable against gram-negative bacteria compared with gram-positive bacteria.

CONCLUSION

The two studied plants (*Origanum majorana* and *Origanum vulgare*) have provided high yields of essential oils respectively 2.50% and 2.34%. This yield has very appreciable economic value. The antimicrobial activity of combination of two essential oils varies according to the studied strains (*Escherichia coli* ATCC25921, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter sp*, *staphylococcus aureus* ATCC25923), but these oils presents a strong activity against all tested bacteria except *Pseudomonas aeruginosae*. This strain showed complete resistance to bioactive substances existing in our extraction product.

The use of synergy of two essential oils can be recommended used for food, phytosanitary, cosmetic and pharmaceutical industries. In addition to its ease of culture, its attractive yield, marjoram and oregano could be developed as part of a pushed valuation policy.

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