

Extraction and evaluation of antibacterial activity of essential oils of oregano (*Origanum vulgare*) in the region of Ouazzane, Morocco

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ABSTRACT

The main objective of this work is to evaluate the antimicrobial activity of the essential oil of oregano (*Origanum vulgare*). *Origanum vulgare* is an aromatic plant, widely distributed in Morocco and widely used by the Moroccan people in order to its medicinal characteristics. *Origanum vulgare* is collected from Ouazzane City in the North of the Kingdom.

The essential oil of oregano is obtained by hydro-distillation in a Clevenger-type apparatus with highest yield leaves up to (2.34%). The inhibitory activity of the essential oil of oregano is not the same against all tested strains (*Escherichia coli* ATCC 25921, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosae* ATCC 27853, *Acinetobacter sp*, *Staphylococcus aureus* ATCC 25923). However, the bactericidal activity was remarkable against all strains tested.

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KEYWORDS

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

INTRODUCTION

The Oregano (*Origanum vulgare*) is a perennial herbaceous, belongs to the family of lamiaceae. This plant of ten has a size ranging from 30 to 60 cm, the stem seems to be hairy and feature rounded green leaves. The flowers are pink or purple and are grouped in to small ears. The oregano plant can be harvested in July. It is known since the dawn of time by his taste and the medicinal properties^[1].

Previously, the using of oregano was widely in medicinal food. The Greeks used the leaves to make poultices to relieving muscle pain. The Romans used

it in cases of snake bites and scorpion. Today, oregano is used as a tea for colds and stimulates digestion. The essential oil of oregano is a powerful antiseptic, but it is also recommended against spasmodic pain fatigue and stress. Its powerful and peppery flavor makes it an important herb in Mediterranean cuisines. Oregano is characterized by the synthesis of odorant molecules characteristic especially essential oils (EO).

It is known by its antiseptic activity^[2,3] and their therapeutic activity in folk medicine^[4]. The essential oils increase the oxygen level and activate the immune system in animal cells^[5]. Furthermore, they

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exhibit an effect on murine cancer cells in culture^[6]. They have a very broad spectrum of inhibition against Gram positive and Gram negative bacteria^[7]. They were considered the most effective antimicrobial agents present in these plants.

For reasons of scientific confirmation, we are interested in the antibacterial effect of the Moroccan species present wildly in the region of Ouazzane (Northern Morocco). This activity will be tested against some strains collected from pathological samples.

MATERIALS AND METHODS

Material

- Plant material

The plant material oregano (*Origanum vulgare*) was collected in July 2012 from the region of Ouazzane; samples were drying in dark 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 nosocomial infections (urinary, intestinal, respiratory, etc.)^[8, 9].

Methods

- The Extraction of essential oils

The extraction of essential oils of oregano (*Orig-*

anum vulgare) was carried out by hydro-distillation in a Clevenger-type apparatus^[10]. 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 dry 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 anhydrous sodium sulfate^[11]. 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^[12]:

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

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

- Microbiological procedure

The used methods are summarized in TABLE 2:

RESULTS AND DISCUSSION

The yield

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. This oil yield obtained is higher than indicated by OJHA et al.^[15].

The author above has shown in a study using the leaves of *Origanum vulgare* in the region of the Indian Himalayas, the essential oil content after extraction was 0,16 to 2.07%. This variation in per-

TABLE 1 : Tested micro-organisms

Microbial group	Tested strains	Origin of strain
	<i>Escherichiacoli</i> ATCC25921	UTI
	<i>Klebsiellapneumoniae</i>	UTI
	<i>Enterobactercloacae</i>	UTI
Gram negative	<i>Pseudomonasaeruginosae</i> ATCC27853	Skin infection
	<i>Acinetobacter sp</i>	UTI
Gram-positive	<i>Staphylococcus aureus</i> ATCC25923	UTI

*UTI: Urinary Tract Infection

TABLE 2 : Methods used in the study of antimicrobial potency

Methods	Used for	Description	Incubation	Play
Technical of the aromatoqram	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 ^[13]	37 °C/ 24	Measurment 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 ^[14]	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/ 18-24h	presence or absence of bacterial growth

TABLE 3 : The essential oil content of *Origanum vulgare*

Plant	Leaves	Stem	Leaves and stem
Quantity	100g	100g	100g
Yield	2.34%	1.10%	0.6%

TABLE 4 : Antimicrobial activity of the essential oil of oregano (*Origanum vulgare*)

Bacteria	Diameter of inhibition Zones (mm)
<i>Klebsiellapneumoniae</i>	14mm
<i>Acinetobacter sp</i>	23mm
<i>Staphylococcus aureus</i>	23mm
<i>Pseudomonas aeruginosae</i>	-
<i>Escherichiacoli</i>	22mm
<i>Enterobactercloacae</i>	17mm

formance can be attributed not only to the origin of the plant and the extraction technique, but also to the collection period of the vegetable plant.

The results of antimicrobial activity of the essential oil of oregano (*Origanum vulgare*) are showed in TABLE 4.

The experimental results presented in TABLE 4 show that the essential oil of oregano 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 essential oil was against *Staphylococcus aureus* and *Acinetobacter sp* (23 mm), followed by the other three strains of *Escherichia coli* ATCC25921 (22mm), *Enterobacter cloacae* (17mm), and *Klebsiella pneumonia* (14mm), these species are sensitive to this EO, while *Pseudomonas aeruginosae* exhibited a resistance to this EO.

According to the inhibition zones generated by

the oil of 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 (*Origanum vulgare*) essential oil are summarized in TABLE 5.

In fact, the essential oil of oregano showed a significant inhibitory effect against microorganisms studied. All microbial strains were inhibited at a concentration of 1/25(V/V). *Escherichia coli* are more resistance to this EO with an MIC of 1/25(V/V). The MIC of *Klebsiella pneumoniae*, *Enterobacter* was the same 1/50 (V/V), where as *Acinetobacter* and *Staphylococcus aureus* have a lowest MIC which was 1/100(V/V). The essential oil of oregano is very active on all strains tested except *Pseudomonas* which was more resistant.

Following these results, the essential oil of oregano has shown very interesting antibacterial

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TABLE 5 : The results of minimum inhibitory concentration (MIC) for the essential oil of oregano (*Origanum vulgare*)

Concentration (V/V) Bacteria	1/10	1/25	1/50	1/100	1/200	1/300	1/500	Control
<i>Klebsiella pneumoniae</i>	-	-	-	+	+	+	+	+
<i>Acinetobacter</i>	-	-	-	-	+	+	+	+
<i>Staphylococcus</i>	-	-	-	-	+	+	+	+
<i>Escherichia coli</i>	-	-	+	+	+	+	+	+
<i>Enterobacter</i>	-	-	-	+	+	+	+	+

-Absence of Growth, +presence of Growth

TABLE 6 : Results of minimum bactericidal concentration (MBC) for the essential oil of oregano (*Origanum vulgare*)

Bacteria	<i>Klebsiella pneumoniae</i>	<i>Acineto-bacter</i>	<i>Staphylo-coccus</i>	<i>Escherichia coli</i>	<i>Enterobacter</i>
CMB (V/V)	1/50	1/100	1/25	1/25	1/50

characteristics against the micro-organisms tested. The results of minimum bactericidal concentration (MBC) of the (*Origanum vulgare*) essential oil are summarized in TABLE 6.

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

CONCLUSION

Origanum vulgare is provided an essential oil with a high yield of 2.34% which is in our opinion has a very significant economic value. The antimicrobial activity of the essential oil is very important against all strains studied (*Escherichiacoli* ATCC25921, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter sp*, *staphylococcus aureus* ATCC25923).

The results showed that there is no any effect against *Pseudomonas aeroginosae*. This strain showed complete resistance to bioactive substances existing in our extraction product.

The findings observed in the essential oil of oregano from Ouazzane allow us to decide the possibility of its using as a natural preservative. It can be recommended for pet food, plant industries, cosmetics and pharmaceuticals. In addition to its ease in culture, its attractive yield, oregano could be developed as part of a policy thrust recovery. *Origanum vulgare* is provided an essential oil with a high yield up to 2.34% which is in our opinion has a

very significant economic value.

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