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# Antibacterial activity of essential oil and some extracts of *Cistus ladaniferus* from Oulmes in Morocco

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## Abstract
The aim of this work was to investigate the antibacterial activity of essential oil and some extracts of *Cistus ladaniferus* collected from Oulmes in Morocco against Gram-positive and Gram-negative bacteria. The antibacterial activity was determined by agar diffusion method and the determination of minimum inhibitory concentration (MIC) was done by microtitration technique. The result showed that the essential oil was active against Gram-positive more than Gram-negative bacteria, the essential oil had strong antibacterial activity against *Staphylococcus aureus* (zone of inhibition of growth is 27 mm) with MIC of 250 µg/ml and *Yersinia enterocolitica* (zone of inhibition of growth is 25 mm) with MIC of 62,5 µg/µl. Methanolic and aqueous extracts had strong antibacterial activity; the maximum zone of inhibition was noted for methanolic extract against *Staphylococcus epidermis* (zone of inhibition of growth is 23 mm) and for aqueous extract against *Staphylococcus epidermis* (zone of inhibition of growth is 20 mm), *Staphylococcus aureus* (zone of inhibition of growth is 20 mm) and *Acinetobacter baumannii* (zone of inhibition of growth is 20 mm). The extracts obtained with dichloromethane, ethyl acetate and hexane inhibited some bacteria and the zone of inhibition of growth were respectively 16 mm, 12 mm and 12 mm against *Staphylococcus aureus*, 13 mm, 11 mm and 8 mm respectively against *Yersinia enterocolitica*. The results indicate that *Cistus ladaniferus* can be used for the treatment of various infections and for the development of new antibacterial agents.

***Keywords:*** antibacterial activity, essential oil, *Cistus ladaniferus*, medicinal plant.

## Introduction
A lot of microorganisms cause some damages for the human health. The abusive use of the antibiotics entailed some resistance at these microorganisms responsible for food toxi-infections. It was therefore very interesting to look for the alternatives for these products [1].
The medicinal plants have been used since the time in order to fight all sorts of infections as the intestinal, cutaneous, respiratory and viral infections [2, 3, 4, 5].
The essential oils of the different medicinal plant species have been used for the assessment of their antibacterial activity and the results proved strong activity against Gram-positive and Gram-negative bacteria [6].
The extracts by different solvents of medicinal plants have also a large specter of activity against the bacteria. Previous study showed that this extracts inhibited most of Gram-positive bacteria, as well as Gram-negative bacteria because her partitions is hard nature then limits the passage of the substances. The medicinal plants could be used for the development of new antibacterial products [7].
*Cistus ladaniferus* is widely distributed in Mediterranean region: in south of Spain, Italy, Sicile, Morocco and Algeria [8]. These shrubs have one to two meters and flowering between April and June. The leaves of all species secrete essential oils and resin. The resin (labdanum) is composed mainly of flavonoides [9, 10, 11].

In Oulmes (middle Atlas), local population use *Cistus ladaniferus* to treat various diseases, this species is frequently used in many traditional medicines for their antimicrobial, antiviral, antitumor, anti-inflammatory, gastric, antioxidant properties [12, 13, 14].
These previous results encouraged us to deepen the studies on antibacterial properties of the essential oil and some extract (hexane, dichloromethane, ethyl acetate, methanol and aqueous) of *Cistus ladaniferus* collected from Oulmes in Morocco against 14 microorganisms.

## Materials and methods

*1. Plant Materials*

*Cistus ladaniferus* (full plant) was collected in May 2008 from Oulmes, was identified by Pr Mohammed Fennane at Scientific Institut of Rabat and was shade dried at room temperature for twenty days.

*2. Plant extracts*

### 2.1. Oil isolation

The essential oil arise from a secondary metabolism of the plant, normally formed in special cells or groups of cells or as glandular hair found on many leaves and steams.
Arial parts of *Cistus ladaniferus* (40%) were cut into small pieces and submitted to hydrodistillation (60% water) for 4 h, according to the standard procedure reported in the European Pharmacopoeia [15].

### 2.2. Organic extracts

Organic extracts (with solvents: Hexane, Dichloromethane, ethyl acetate and methanol) was obtained by Soxhlet extraction of 200g of powder of *Cistus ladaniferus* for 24 h in 800 ml of solvent used. The extract obtained was concentrated to dryness and the residue was kept at 4°C.

### 2. 3. Aqueous extract

10 g of powder of *Cistus ladaniferus* were extracted with boiling water (100 ml) for 30 min; the decoction was filtered and then freeze-dried.

*3. Microorganisms used*

The test organisms used included 14 bacteria strains: *Streptococcus sanguins, Staphylococcus epidermis, multiresistant Staphylococcus aureus, Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas fluoresce, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella Typhimurium, Salmonella arizonae, Hafnia alveie, Yersinia enterocolitica, Escherichia coli and Klebsiella pneumoniae*. These strains were collected from the National Institute of Health (NIH) Rabat-Morocco.

*4. Antibacterial assay*

### 4.1. Zone of inhibition

Antibacterial activity was assayed by disc diffusion method described by Nongponga et al [16]. The essential oil was diluted with Tween 80; Hexane, Dichloromethane and Ethyl Acetate extracts were diluted with DMSO (Dimethylsulfoxide) ; Methanol and aqueous extracts were diluted with sterile distilled water.
Muller Hinton agar was used for this study; bacterial cultures freshly grown at 37° C/24 h were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 105 CFU: ml. The organisms were spread on Muller Hinton plates by cotton swab, wells of 6 mm diameter were pouched into the agar medium filled with 50µl of different plant extracts. The plates were incubated for 24 h at 37°C and antibacterial activity was evaluated by measuring the inhibition zone diameter against the test organism.

### 4.2. Minimum Inhibitory Concentration (MIC)

The determination of MIC of differents extracts from *Cistus ladaniferus* against bacterial strains was performed according to the microtitration technique described by Eloff [17].

## Results and discussion

The result of bacterial activity of the different extracts of *Cistus ladaniferus* are indicated in table 1. The results have showed that the essential oil has very good activity against all bacteria: Gram-positive more than Gram-negative bacteria. The essential oil of *Cistus ladaniferus* has maximum zone of inhibition against *Multiresistant Staphylococcus aureus* (28 mm) and *Staphylococcus aureus* (27 mm see fig 1 photo 3) while

the minimum zone of inhibition was against *Salmonella enteritidis* and *Pseudomonas aeruginosa* (12 mm), the most significant results were recorded for the dilutions of the order 1/2.

**For essential oil :**

**Photo1:***Klebsiella pneumoniae*

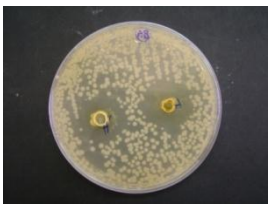

**Photo2:***Acinetobacter baumannii*

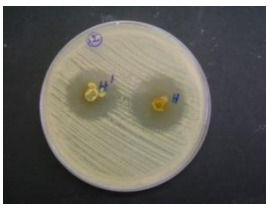

**Photo3:***Staphylococcus aureus*

**For methanolic extract :**

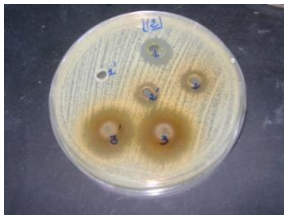

**Photo4:***Staphylococcus aureus*

**Photo5:***Streptococcus sanguins*

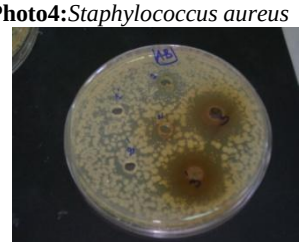

**Photo6:***Acinetobacter baumannii*

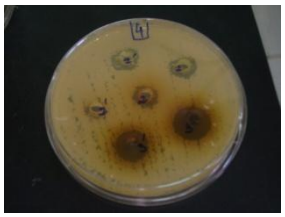

**Photo7:***Escherichia coli*

**Photo8:***Pseudomonas fluoresce*

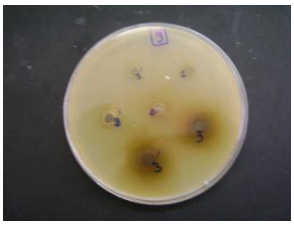

**Photo9:***Salmonella Typhimurium*

**Figure 1:** Agar-well diffusion assay: zone of inhibition of growth of some strains bacteria.

**Table1**: Screening of antibacterial activity of *Cistus ladaniferus* extracts collected from Oulmes in Morocco (Diameter of zone of inhibition is in mm)

| | **EO** | | | **Hexane Ext** | | | **EtOAc Ext** | | | **MeOH Ext** | | | **DCM Ext** | | | **$H_2O$ Ext** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dilutions / Strains | 1/2 | 1/4 | 1/8 | 1/2 | 1/4 | 1/8 | 1/2 | 1/4 | 1/8 | 1/2 | 1/4 | 1/8 | 1/2 | 1/4 | 1/8 | 1/2 | 1/4 | 1/8 |
| **SS** | 20 | 19 | 18 | 11,5 | 10 | - | 13,5 | 11 | 8 | 21,5 | 19 | 15 | 12 | 11 | _ | 15 | 12 | 11 |
| **SE** | 25 | 23 | 19 | 10,5 | 8 | - | 11,5 | 10 | 8 | 23 | 19 | 17 | 16 | 15 | 12 | 20 | 18 | 15 |
| **SA** | 27 | 25 | 22 | 12 | 10 | - | 12 | 10 | 8 | 21 | 19 | 15 | 16 | 15 | 13 | 20 | 17 | 15 |
| **MSA** | 28 | 25 | 21 | - | - | - | 13 | 11 | 8 | 19 | 17 | 16 | - | - | - | 15 | 14 | 11 |
| **SL1** | 12 | 11 | 9 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **PA** | 12 | 10 | 10 | - | - | - | 12 | 11 | - | 21 | 18 | 14 | ND | ND | ND | ND | ND | ND |
| **HA** | 13 | 10 | 9 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **SL3** | 15 | 14 | 12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **KP** | 15 | 14 | 14 | - | - | - | - | - | - | 17 | 15 | 13 | - | - | - | 16 | 15 | 12 |
| **EC** | 18 | 17 | 17 | 10 | 8 | 8 | 10 | 8 | - | 15,5 | 12 | 10,5 | ND | ND | ND | ND | ND | ND |
| **SL2** | 19 | 17 | 12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **PF** | 22 | 20 | 19 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **AB** | 24 | 20 | 18 | - | - | - | - | - | - | 20 | 19 | 13 | 18 | 17 | 13 | 20 | 19 | 15 |
| **Y** | 25 | 22 | 19 | 8 | - | - | 11 | 10 | - | 21 | 18 | 15 | 13 | 11 | - | 18 | 17 | 13 |

***SS** :Streptococcus sanguins, **SE** :Staphylococcus epidermis, **SA** :Staphylococcus aureus, **MSA** :multiresistant Staphylococcus aureus, **SL1** :salmonella enteritidis, **PA** :Pseudomonas aeruginosa, **HA** :Hafnia alveie, **SL3** :Salmonella*

*arizonae, **KP** :Klebsiella pneumoniae, **EC** :Escherichia coli, **SL2** :Salmonella Typhimurium, **PF** :Pseudomonas fluoresce, **AB** :Acinetobacter baumannii, **Y** :Yersinia enterocolitica.*
*EO: Essential oil; Hexane Ext: Hexane extract; EtOAc Ext: Ethyle acetate extract; MeOH Ext: Methanol extract; DCM Ext: Dichloromethane extract; $H_2O$ Ext: Aqueous extract; 1/2 1/4 1/8: Dilutions; ND: no determinate; - Bacterial growth.*

The methanolic extract studied showed inhibition of growth of the tested microorganisms with various degrees, the result showed that the extract exhibited high antibacterial activities against (9/14) strains tested and the inhibition diameter of this extract was between 15.5-21.5 mm with average 13 mm. The results of aqueous extract showed inhibition of growth of 7/12 strains tested with inhibition diameter between 15-20 mm (average 10 mm). For dichloromethane extract the result showed that this extract has a medium activity with inhibition diameter between 12-18 mm (5/12 strains tested), the ethyl acetate extract has inhibition diameter between 10-13.5 mm (7/14 strains tested) and for hexane extract the inhibition diameter was between 8-12 mm (5/12 strains tested).

Minimum inhibitory concentration (MIC) result of essential oil of *Cistus ladaniferus* was presented in table 2, the essential oil showed MIC between 50-500 µg/ml. The higher MIC against tested bacterial strains was for *Multiresistant Staphylococcus aureus* (MIC was 50 µg/ml) and for *Yersinia enterocolitica* (MIC was 62.5 µg/ml).

**Table2**: Minimum inhibitory concentration of *Cistus ladaniferus* extracts collected from Oulmes in Morocco

| Strains | **SS** | **SE** | **SA** | **MSA** | **SL1** | **PA** | **HA** | **SL3** | **KP** | **EC** | **SL2** | **PF** | **AB** | **Y** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MIC (µg/ml) | ++ | ++ | + | +++ | - | ++ | + | + | ++ | + | + | - | + | +++ |

***SS** :Streptococcus sanguins, **SE** :Staphylococcus epidermis, **SA** :Staphylococcus aureus, **MSA** :Multiresistant Staphylococcus aureus, **SL1** :salmonella enteritidis, **PA** :Pseudomonas aeruginosa, **HA** :Hafnia alveie, **SL3** :Salmonella arizonae, **KP** :Klebsiella pneumoniae, **EC** :Escherichia coli, **SL2** :Salmonella Typhimurium, **PF** :Pseudomonas fluoresce, **AB** :Acinetobacter baumannii, **Y** :Yersinia enterocolitica.*
MIC: Minimum inhibitory concentration; +: 250µg/ml; ++: 125µg/ml; +++: 50µg/ml; - : No activity.

Many microorganisms which cause damage to human health, the increasing trend of resistance to the antibiotics in current use has drawn the attention of researchers to natural alternative treatments of bacterial infections as potential sources of new antimicrobial agents. *Cistaceae* (rock-rose or rock rose family) is one of family plants used in the medicine; many studies have reported many of biological activity of extracts of *Cistus* [18]. In this work, the Gram-positive bacteria showed more sensibility for different plant extracts than Gram-negative bacteria [19], the structure of cellular wall of Gram-positive bacteria was haw ever more sensitive to the action of this extract [20], this is credited to the presence of outer membrane lipopolysaccharides [21 22].

Our result showed that the essential oil of *Cistus ladaniferus* from Oulmes has strong activity against strains Gram-positive bacteria *Multiresistant Staphylococcus aureus* with MIC 50µg/ml and against strains Gram-negative bacteria *Yersinia enterocolitica* with MIC 62.5µg/ml. The antibacterial activity of essential oil from Commercial units (Distillery" U Mandriolu "and" Phytosun ") was found to be the most active against *Staphylococcus aureus* with MIC 200µg/ml [23] when our essential oil has 250 µg/ml against same strains.

Mohammedi [24] studied antibacterial activity of essential oil from Algeria and showed that no zone of inhibition has been observed around the disks of 3 µl with natural essential oil after incubation of the bacterial cultures of *Citrobacter* and *Pseudomonas aeruginosa*, when with essential oil from Oulmes (Morocco) in our study we have a total inhibition of the bacterial growth of all strains bacteria used (the low zone is in order of 12 mm for *Salmonella entertidis* and most elevated is in order of 28 mm for *Multiresistant staphylococcus aureus*).

The highest activity with the essential oil has been marked against *Listeria monocytogenes* with average of zone of inhibition 19.5 mm, when a lowest is with average of zone of inhibition 10.66 mm against *Staphylococcus aureus*; *Klebsiella pneumoniae* and *Salmonella thyphi* appeared insensible against the action of this essential oil.

Antibacterial activity obtained in this study varied with solvents used for extraction. Methanolic extract exhibited higher activity towards most the strains tested, the maximum activity was recorded against *Staphylococcus epidermis*, *Streptococcus sanguins* (see fig 1 photo 5), *Staphylococcus aureus* (see fig 1 photo 4), *Pseudomonas aeruginosa*,, *Yersinia enterocolitica*, *Acinetobacter baumannii* (see fig 1 photo 6) and *Multiresistant Staphylococcus aureus*, the diameter of inhibition were respectively 23, 21.5, 21, 21, 21, 20 and

19mm. It could be noted that for the different extracts of *Cistus ladaniferus* antibacterial activity has been marked more for Gram-positive than Gram-negative strains bacteria. According to the results achieved for study of the antibacterial activity of methanol ethanol and ethyl acetate extracts of *Myrtus communis Cistus ladaniferus* and *Cistus monopeliensis*, Gram-positive strains were more sensitive to these extracts than Gram-negative strains bacteria tested [25], and it's agrees with our results, the aqueous extract of *Cistus ladaniferus* of Oulmes has antibacterial activity against 4/4 strains Gram-positive tested and 3/8 strains Gram-negative bacteria tested, the diameter of inhibition were proximate than those obtained of methanol extract.
For hexane, dichloromethane and ethyl acetate extracts we observed low diameters of inhibition compared with precedent results, ethyl acetate extract inhibited 7/14 with average 12 mm, dichloromethane extract inhibited 5/12 with average 15 mm and hexane extract inhibited 5/14of growth of bacteria strains tested with the lowest diameter of inhibition and with average 10.5 mm.
The antibacterial activity demonstrated by the different types of extracts may be attributed to the diversity of structures or the uneven distribution of chemical constituents within this extract, *Cistus ladaniferus* is rich in polyphenolic terpenes and flavonoids compounds [26. 27].
Bouamama et al., studied antibacterial activity of the leaf extract of another species of *Cistaceae* : *Cistus villous* and showed that methanol ethyl acetate butanol and aqueous extract has the lowest and therefore the most interesting MIC values (1.56 mg/ml) against four strains bacteria [28].
In our experiment we also found that according in the increasing order of the polarity of the solvents extraction, the antibacterial activity was to go better, so for hexane ethyl acetate and methanol extract we found respectively antibacterial activity against 5/14 7/14 and 9/14 strains bacteria tested.

## Conclusion

The various traditional uses of the majority of tested plants correlate well our findings. The results of this preliminary evaluation give evidence that some of the ethnobotanically selected and traditionally used moroccan plants species can be regarded as promising resources for antibacterial, our results suggest that *Cistus ladaniferus* has antibacterial activity against Gram-positive and Gram-negative strains bacteria and we affirmed that Gram-positive bacteria were more sensitive. It's first time in our knowledge the antibacterial activity of *Cistus ladaniferus* from Oulmes in Morocco was evaluated against strains bacteria causes some infections nosocomiales (*Acinetobacter baumannii, multiresistant Staphylococcus aureus*), and responsible of the food infections (*Yersinia enterocolitica*) and urinary infection (*Staphylococcus aureus, Klebsiella pneumoniae*).
Further research is needed toward isolation and identification of active principles present in the different extracts which could possibly be exploited for pharmaceutical use.

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