

Antibacterial activity of essential oils from *Cistus ladaniferus* L. and *Lavandula stoechas* L.

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Abstract: Since antiquity, the plants are known for their medicinal properties. It's why we are interested to extract essential oils from two plants known by the rural population by their properties of good disinfecting from the wounds. These plants are *Cistus ladaniferus* L. and *Lavandula stoechas* L. which growth spontaneously and with abundance in the western north of Algeria. The volatile oils extracted by hydrodistillation were tested in vitro for evaluate their capacity to inhibit the pathogenic germs growth. Variable antibacterial activities were noted; whereas a remarkable activity was recorded against the strain *Listeria monocytogenese* ATCC 19111 exerted by the oil components of *C. ladaniferus* where the IC₅₀ is about 1.36 µl/ml.

Keywords: *Cistus ladaniferus*; *Lavandula stoechas*; essential oil; antimicrobial activity.

Introduction

The infections risk related to pathogenic germs increases at the present time considering the increased resistance which certain microbes acquire, whose usual antibiotics are ineffective to treat the infectious disease. This is why that many diseases which we controlled formerly, have reappeared and escaped from human control. The antibacterial activities of essential oils from various medicinal plants against micro-organisms were described and proven in experiments by various researchers.^{1,2,3}

Cistus ladaniferus from the Cistaceae family and *L. stoechas* from the Lamiaceae family are known in medicinal traditions by their properties to disinfect wounds. This is why a great interest of our share it is directed towards these abundant and not cultivated plants, growth to the wild state and adapt easily to the Mediterranean climate especially in Algeria.

In the essential oil of *L. stoechas*, 51 compounds have been described, the major ones being fenchone, pinocarvyl acetate, camphor, eucalyptol and myrthenol constituting 63.4% of the oil, whereas the studies

carried out on the *Cistus* showed the presence of pinene, camphene, myrcene, phellandrene, limonene, thujone, citral and geraniol in volatile oils.^{4,5}

Plant material

Lavandula stoechas and *Cistus ladaniferus* were collected from Tlemcen mounts (Algeria).

Extraction method

Essential oils of each plant were isolated from 200g dry leaves by hydrodistillation in a Clevenger- type apparatus during 3 hours.

Bacteria strains

The test was carried out on bacteria isolated from the hospital medium (IS) and on reference strains. *Pseudomonas aeruginosa* ATCC27853, *E. coli* ATCC25922, *Staphylococcus aureus* ATCC25923 (Laboratory of Antibiotics, Antifungal, Physico-chemistry, Synthesis and biological Activity, Tlemcen - Algeria), *P. aeruginosa* (IS), *Enterobacter cloacea* (IS), *Klebsiella pneumoneae* (IS), *E. coli* (IS),

Staphylococcus aureus ATCC601 (Laboratory of phytopharmacology, Paris 7 - France), *Salmonella typhi* (IS), *Listeria monocytogenes* ATCC 19111 (Laboratory of microbiology, Tlemcen - Algeria), *Proteus mirabilis* (IS), *Citrobacter sp.* (IS).

in vitro antibacterial assay

The antibacterial assay was carried out by diffusion method on solid medium⁶; the culture medium used is Muller Hinton⁷. 10⁶ cfu of each pure culture from 24 hours was spread out over medium Mueller Hinton, Petri box (9 cm in diameter) were filled preliminary with 10ml of the medium, discs of 6mm in diameter charged with 3µl of essential oil were deposited on the medium surface. For each bacteria and oil, the test was repeated three times. Controls without essential oil were carried out. Petri box were incubated at 37°C during 20 hours.

Growth study by measurement of optical density

Increasing volumes of essential oil were added in the liquid culture medium Mueller Hinton to study the sensitivity of the microbial strain with respect to the increasing concentrations of oil. Into tubes containing 10ml liquid medium, various quantities of oil were introduced under conditions sterile, after a good agitation, into each tube we introduced 10⁶ cfu of a pure culture. Incubation was carried out at 37°C during 20 hours. After incubation, measurements of growth density in each tube were carried out against a blank. The reading was made by a 6405UV/Vis spectrophotometer at 625 nm. The percentage of microbial growth inhibition for each concentration was measured by the following formula:

$$\text{Inhibition\%} = (\text{A control} - \text{A test}) / \text{A control} \times 100$$

A control: optical density at 625nm of control without extract

A test: optical density at 625nm of sample test

The values obtained were exploited to plot a curve whose ordered represents the growth inhibition of the germ expressed as a percentage and the axis represents the amount of essential oil in medium. Of this curve, we determined the concentration of oil necessary to reduce at 50% the microbial growth.

Results and discussion:

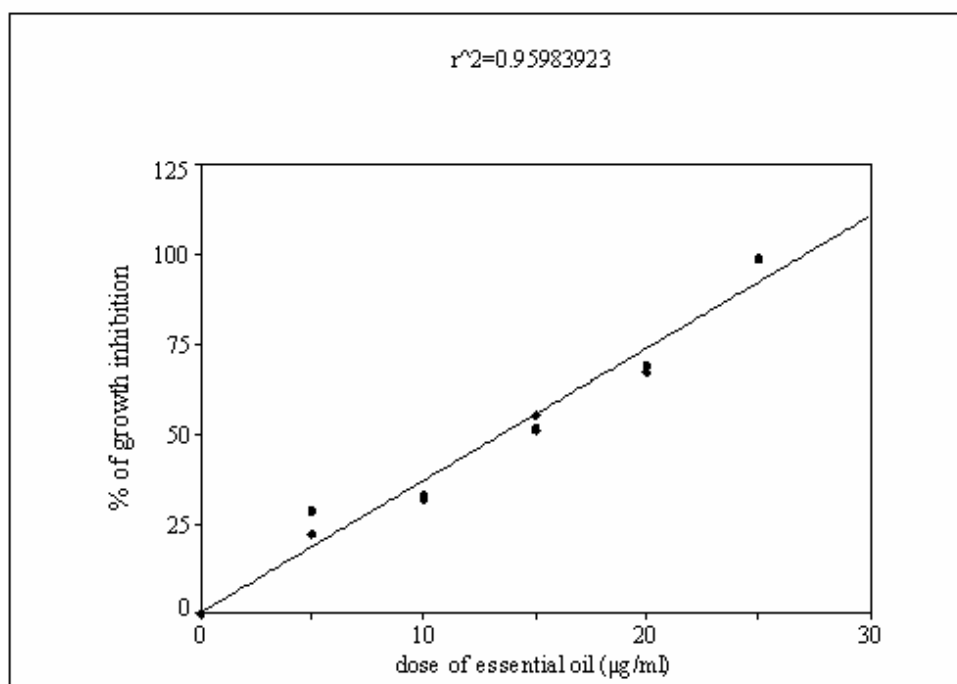
No inhibition zone was observed around discs to the amount of 3µl/disc after incubation of bacterial cultures from *Citrobacter sp* (IS) and *P. aeruginosa* (IS). These strains have a high resistance potential against antimicrobial action of these two oils.

On the other hand variable activities whose diameters of inhibition zones have not exceeded 20 mm were recorded against other bacterial strains (Table 1). The good and highest activity is that exerted by *Cistus* oil against *L. monocytogenes* ATCC 19111 whose average value of the inhibition zone was 19,5mm. Also noting a zone of 10.66 mm on average with this same oil against *S. aureus* ATCC 601. We also noticed that *K. pneumoneae* (IS) and *S. typhi* (IS) were insensitive against the activity of *Cistus* essential oil.

The high antimicrobial capacity of Lavender was observed against *E. coli* ATCC but whose diameter is only a few millimetres; approximately 8.19mm. A very effective antibacterial capacity was noted and proven in vitro against *S. aureus*, *E. coli*, *K pneumoniae* and *P. aeruginosa* by Gören and al.⁸

Table 1: Antimicrobial activity of essential oils expressed or represented in zones of inhibition (mean ± SD) in mm diameter

Strain	<i>L. stoechas</i>	<i>C. ladaniferus</i>
<i>P. aeruginosa</i> (SI)	0	0
<i>P. aeruginosa</i> ATCC27853	0.52 ± 0.3207	0
<i>E. cloacea</i> (SI)	2.86 ± 0.9942	3.22 ± 1.619
<i>K. pneumoneae</i> (SI)	5.88 ± 0.5728	0.33 ± 0.2887
<i>E. coli</i> ATCC25922	8.19 ± 1.496	4.67 ± 0.7638
<i>E. coli</i> (SI)	2.83 ± 0.8780	2.67 ± 0.2887
<i>S. aureus</i> ATCC25923	5.90 ± 1.670	3.5 ± 1.323
<i>S. aureus</i> ATCC 601.	6.83 ± 1.443	10.66 ± 1.155
<i>S. typhi</i> (SI)	3.67 ± 1.155	0
<i>L. monocytogenes</i> (SI)	5.64 ± 0.7743	19.5 ± 0.5000
<i>P. mirabilis</i> (SI)	5.66 ± 1.258	3.83 ± 1.528
<i>Citrobacter sp</i> (SI)	0	0

Figure1: Effect of *Cistus ladaniferus* on the growth of *L. monocytogenes* strain

The results obtained by discs method made it possible to select *Cistus* essential oil and *L. monocytogenes* ATCC 19111 strain to carry out another complementary antimicrobial test, can be summarized by the monitoring of the increase amount effect of oil on the growth density of the micro-organism population. Measurements of microbial density read at 625 nm were transformed and expressed as a percentage of microbial population inhibition. From these values, we drew up a curve (Figure 1) illustrating the growth reduction and the significant sensitivity of the germ with respect to the active substances from *Cistus*.

A reduction proportional to the amount of the oil was recorded whose concentration which inhibits 50% microbial population or IC50 is about 1.36 µl/ml ±

0.0037. This antimicrobial activity is not related only to the presence of a particular substance but, it is the resultant of the complex action of various aromatic structures from oil.

In the conclusion, the plants are a reserve of biologically active substances. Essential oils can be a significant source of a great diversity of chemical species equipped with antimicrobial capacity, the oil of *Lavandula stoechas* and *Cistus ladaniferus* can have application in therapy of the infectious diseases is like substituents of certain antibiotics or like complementary agents used in synergy with the synthesis substances. Essential oils can also have application in food industries not only like aromatizing but also like preservative of foodstuffs.

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