JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *CISTUS LADANIFER* VAR. *MACULATUS* DUN

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doi: 10.15414/jmbfs.2018-19.8.3.925-930

ARTICLE INFO

Received 9. 3. 2018
Revised 20. 9. 2018
Accepted 5. 10. 2018
Published 1. 12. 2018

Regular article

OPEN ACCESS

ABSTRACT

The aim of the present study was to identify chemical composition and evaluate antimicrobial effectiveness of the essential oil of *Cistus ladanifer* var. *maculatus* Dun. The essential oil of C. *ladanifer's* leaves was obtained by hydro-distillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS). 28 compounds were identified, representing 88.67% of the total constituents. The main compound was identified as Verticiol (18.16%), followed by Camphene (17.70%), γ-Gurjunene (7.15%), n-Butylcyclohexane (5.95%) and Bornyl acetate (5.86%). The oil was found to be rich in Monoterpene Hydrocarbons (32.05%), followed by Diterpenic alcohol (18.16%), Oxygenated hydrocarbons (13.27%), Sesquiterpene hydrocarbons (12.40%), Monoterpenic ester (5.86%), Cyclic ether (4.36%) and Oxygenated sesquiterpenes (2.57%).
Antimicrobial activity of the essential oil of *C. ladanifer* was tested against two Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis)* and two Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, and three fungi (*Candida albicans*, *Trichophyton rubrum* and *Aspergillus niger*), by the microdilution method. In fact, strong inhibitory and bactericidal effect has been shown against all tested Gram-positive and Gram-negative bacteria. Indeed, strongest inhibitory and fungicide effect was exerted against the yeasts and molds studied.
It seems following this study that the essential oil of *C. Ladanifer* has very significant antimicrobial activity, it shows a broad spectrum covering Gram-positive, Gram-negative bacteria, yeasts and fungi.

Keywords: *Cistus ladanifer* var. *maculatus* Dun., Cistaceae, essential oil, composition, gas chromatography, Verticiol, Monoterpene Hydrocarbons, antimicrobial activity

INTRODUCTION

For centuries, medicinal plants have been used as a remedy for various diseases. Plants have the capability to synthesize a wide range of phytochemicals that possess significant biological functions. Many of these compounds have beneficial effects on human health and can be used to treat diseases **(Mahesh and Satish, 2008; Prasannabalaji et al., 2012)**.
Moreover, plants have the ability to synthesize aromatic substances, many of which are bioactive phytochemicals **(Cowan, 1999)**. Over the past few years, the use of plant based natural antimicrobials in the treatment of bacterial infections has gained much recognition **(Kasrati et al., 2014)**.
Furthermore, antimicrobial resistance is now a global concern, which reached a crisis point in many hospitals worldwide. Indeed, there is an urgent need to replace our arsenal of anti-infective agents **(Cushnie, 2011)**, by developing new antimicrobial agents. In this context, a great interest has been focused on the natural products, in particular essential oils **(Bakkali et al., 2008)**. One of their apparent properties is their antiseptic power, linked to their activity against pathogenic bacteria, including some antibiotic resistant strains. Several previous data already described many recipes of herbal and aromatic oils that priests and doctors employed. Currently, the use of essential oils is carried out on scientific and rational basis, in order to develop new products for various fields: food, medical, veterinary and cosmetic.
Hence, several research studies have proved the antimicrobial, antiviral and insecticide properties of terpenes **(Salah-Fatnassi et al., 2010; Nsambu et al., 2014)**. In addition, chemical complexity of the essential oils prevents the decoding from pathogens and thus reduces the risk of developing microbial resistance.

Sixteen species of the genus *Cistus* (Cistaceae) are known to be native in the Mediterranean area's flora **(Gültz et al., 1996)**. Leaves of all *Cistus taxa* are covered with glands secreting resin and essential oil. Plants essential oil and resin consist mainly of terpenoids **(Gültz et al., 1984; Demetzos et al., 1994c)**, flavonoid aglycons **(Vogt et al., 1987b; Demetzos et al., 1990)**, and glycosides **(Vogt et al., 1987a)**. Labdane diterpenes, which appears to be the predominant compounds of the essential oils have been studied for their antimicrobial and cytotoxic activity **(Chinou et al., 1994; Demetzos et al., 1994a; Demetzos et al., 1994b; Dimas et al., 1998; Anastassaki et al., 1999)**. In the family Cistaceae, *Cistus ladanifer* is the most important species used in the perfume and cosmetic industries **(Mariotti et al., 1994)**. *C. ladanifer* is a very poorly exploited species in Morocco for its aromatic virtues; although it is famous on the Mediterranean market (Spain and France). Indeed, Spain is the main producer of the oil which is obtained from the leafy branches, while labdanum extracts are obtained from the gum **(Simon-Fuentens et al., 1987)**. *C. ladanifer* is represented in Morocco by two varieties which differ primarily by the flowers petal color: *C. ladanifer* var. *albiflorus* Dun with completely white petals and *C. ladanifer* var. *maculatus* Dun with mottled petals of crimson. This present study was performed on var. *maculatus* which grows spontaneously in the Moroccan Middle Atlas.
Many chemical studies have been done on the essential oil composition of *C. ladanifer* **(Garcia-Martin & Garcia-Vallejo,1969; Konigs and Gulz, 1974; Proksch et al., 1980 a, b; Gulz et al., 1984; Peyron and Alessandri, 1986; Simon-Fuentes et al., 1987; Mariotti et al., 1997; Weyerstahl et al., 1998; Ramalho et al., 1999)** because of its great interest to the perfume industry. Recently, reports on antimicrobial activity of the plant mentioned and reports on chemical composition refer to samples from Spain **(Fernández-Arroyo et al., 2010; Barrajón-Catalán et al., 2010, 2011)**, Portugal **(Ferreira S et al., 2012; Barros L et al., 2013; Delgado F et al., 2016)**, France **(Christine Robles et al.,**

2003) and Morocco **(Mrabet et al., 1999; Greche et al., 2009)**. As far as we know there is no report on the chemical study and biological properties of *C. ladanifer* essential oils, from our study area except a few attempts have been reported by **Benayad et al., 2013**.
In this study, we examined the chemical composition of volatile components and antimicrobial activity of the leaves oil. The volatile components were identified using GC/MS. The antimicrobial activity was studied by microdilution methods against 7 selected degrading and pathogenic microorganisms.

MATERIAL AND METHODS

Plant material

The aerial part (stems, leaves) of *C. ladanifer* var. *maculatus* Dun were collected in August 2013 in the region of Harcha (Oulmes, Morocco). The Harcha forest spreads out on a mountainous area. It is characterized by a subhumid region, surrounded by a semi-arid zone and is located between 600 and 1300 m of altitude **(Boudy, 1951)**. The botanical identification was performed by Dr. Ahmed BOUKIL (Chief Engineer of Water and Forests, UNDP consultant).

Isolation of essential oil

From fresh leaves of *C. ladanifer* var. *maculatus* Dun, 200g was hydrodistilled for 5 hours using a Clevenger-type apparatus. The essential oil was collected, dried over anhydrous Na_2SO_4, and was then kept in dark at 4°C until further use.

Chemical analysis

Analysis of the volatile compounds was performed on Hewlett-Packard gas chromatograph/mass spectra (GC/MS) system. The chromatograph in split ratio (1:30) mode was fitted with a DB-5 fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 mm). The carrier gas was He with a flow rate close to 2 mL/min. The injector and detector temperatures were 250°C and 280°C, respectively. The column temperature was programmed as follows: 50°C (10 min), 50-190°C at (2°C/min) and 190°C (10 min). The mass spectra were recorded on a selective quadrupolar type detector of 5772 class, ionization was obtained by electronic impacts under a potential of 70 eV. The mass range was between m/z 35-425 *amu*, while scan time was 0.2 scan/s. Compounds were identified by their mass spectra and retention indices. Quantitative analysis was conducted on a HP 5890 gas chromatograph fitted with a flame ionization detector. The column and conditions of use were the same; although N was used as the carrier gas.
The individual compounds were identified by MS and their identity was confirmed by comparing their Kováts Index (KI), their mass spectra and retention times with those of authentic samples or with data already available in the NIST library and in the literature **(Adams, 2007)**.
The KI of a compound is a number obtained by interpolation, relating the adjusted retention time of the sample compound to the adjusted retention times of two standards (in our analysis C8 and C32) eluted before and after the sample compound peak.
The NIST employed was a compilation of 191,000 spectra, 163.000 chemical structures, 121,000 Kováts retention indices and 5200 MS/MS spectra.

Microorganisms tested

To evaluate the antibacterial and antifungal activity of essential oil of *C. ladanifer*, tested bacteria include four strains in which *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. These strains were collected from samples of different pathological origins, from patients consulting in the regional hospital of Meknes city. The tested fungi, included *Candida albicans*, *Trichophyton rubrum* and *Aspergillus niger* which were collected from the Military Hospital of Meknes city.

Inocula preparation

Before use, bacterial strains were revivified by subcultures in Luria-Bertani (LB) plates at 37°C for 24h. Stock bacterial inocula suspensions were obtained from 18 hours culture on Mueller Hinton broth at 37°C. Those final suspensions served for the inocula preparation. The cell density of each suspension was determined by using a counting chamber, and then adjusted to a concentration of 10^5-10^6 CFU/mL by dilution with Mueller Hinton broth.
Revivification of fungal was made by subcultures in malt extract agar plates (malt extract 30g/L and agar 20g/L) at 25°C for 7 days. After incubation, their spores were harvested by scraping the culture surface in sterile NaCl solution 0.9%. Then the spore suspension was concentrated by centrifugation at 10000g for 15 min at 4°C until a concentration of 10^6 spores/mL (counted with a hemocytometer). While, yeast strains were inoculated in yeast peptone-glucose agar (YPG) and incubated at 30°C for 48 h.
A. niger and *T. rubrum* were grown on Sabouraud agar at 35°C for 7 days, to induce conidia formations. Then, the culture was washed with 2 mL of saline solution (0.9% NaCl) and the suspension was transferred to a sterile tube, where the heavy particles were allowed to settle for 5 min. The upper homogeneous suspensions were transferred with a pipette Pasteur to a new sterile tube and filled with Sabouraud broth, then vortexed for few seconds. The mixture of conidial and hyphal fragments was filtered with Wattman filter, which retains hyphal fragments and permits the passage of only *T. rubrum* and *A. niger* microconidia; this suspension is considered as the initial inoculum. After dilutions in Sabouraud broth, the inoculum was adjusted microscopically to about 10^4 CFU/mL.

Evaluation of antimicrobial activity by diffusion method in agar medium

The technique used is a modification of the method of **Fauchère and Avril, (2002)**. It consists of depositing sterile disks of filter paper (Whatman N° 40) 6 mm in diameter soaked with a decreasing concentration range of essential oil 20, 10 and 5µL solubilized in the Tween 20% deposited on a microbial mat and then measure the area where microorganisms could not develop.
A disk soaked with 20 µL Tween 20% was used as negative control on the same plate; Gentamicin® was used as a standard antibiotic for bacterial strains with a concentration of 0.04 g/mL and Canaflucon (Fluconazole)$^{Win®}$ capsule 150 mg with a concentration of 0.6 µg/mL was used as a positive control for fungal strains.
The dishes are left for 1 hour at room temperature to allow the diffusion of the essential oil and are then incubated at 37°C, for 18 to 24 hours for the bacterial strains and 48 hours for the fungal strains. After incubation, the inhibition diameter, translating the antimicrobial activity of the essential oil, is thus determined as a translucent halo around the disc which is measured in millimeters, disc included.

Determination of minimum inhibitory concentration against bacteria

A microdilution of the tested essential oil is produced in a microplate containing 20 µL of Tween 20%, so as to generate a base dilution range 2. The concentration range is then produced in the 96 wells (Greiner, VWR).
20 µL of the essential oil is added to the first well of each line from which a basic geometric dilution 2 is carried out. Then 160 µL of Mueller Hinton broth (MHB) inoculated with 20 µL of a bacterial suspension at 10^6 CFU/mL; are added to each well. Each row is reserved for a particular strain, this work was carried out on four strains and therefore on four rows. Wells containing MHB inoculated with the determined strain are used as a positive control; those containing Tween 20% and non-inoculated MHB are used as a negative control. After an incubation time of 18h, the minimum inhibitory concentration (MIC) of the essential oil is deduced from the first well of the range devoid of microbial growth. The reading is carried out using a 2,3,5-diphenyltetrazolium chloride [TTC, red tetrazolium] colored indicator diluted in sterile distilled water to the order of 0.2 g/mL, by addition of 40 µL of TTC followed by an incubation for 30 min at 37°C, TTC reveals the presence of living bacteria by the appearance of a red coloration **(Eloff, 1998)**.

Determination of Minimum inhibitory concentration against fungal strains

To investigate the antifungal activity of the studied essential oil against *A. niger* and *T. rubrum* a modified microdilution technique described by **Daouk et *al.*, (1995)** was used. Firstly, 50 µl of malt extract broth were added from the second to the 12th well. The essential oil was diluted in Tween 20 % (v/v) at a final concentration of 40 % (v/v), then 100 µL of this solution were deposed in the first well. Afterwards, scalar dilution was made by transferring 50 µL from the 1st to the 11th well. The 12th well was considered as growth control. Thereafter 50 µL of the fungal spore suspension was added to each well to reach a final concentration of 10^6 spores/mL. The microplate was sealed and incubated for 72 h at 30°C. The lowest essential oil concentration that prevents visible fungal growth was defined as the MIC. Likewise, the MIC determination against *C. albicans*, was performed in 96 well-microplates according to the protocol previously described with slight modifications of **Chraibi et al. (2016)**. The essential oil was also serially diluted in YPG broth supplemented with agar at 0.15% (w/v). The 12th well was also considered as growth control. Then, 50 µL of fungal inoculum were added to each well at a final concentration of 10^3 CFU/mL. Finally, the microplate was sealed and incubated at 30°C for 48 h. Experiments were carried out in triplicate. Similarly, the lowest essential oil concentration that prevents visible fungal growth was defined as the MIC.
MIC was detected by lack of visual turbidity (matching the negative growth control). Subcultures were made from the clear wells which did not show any

growth after incubation during the MIC assays on Mueller Hinton agar for bacteria and Sabouraud agar for fungi. **Minimum Bactericidal Concentration (MBC)** or **Minimal Fungicidal Concentration (MFC)** is calculated by transporting 10 µL of the wells showing no microbial growth on a solid medium. The lowest concentration of an antibacterial drug results in a 99.9% reduction of the initial microbial density. EP routinely performs antimicrobial effectiveness testing services which normally includes MBC (minimum bactericidal concentration)/MFC (minimum fungicidal concentration) in conjunction with MIC (minimum inhibitory concentration) to distinguish if antimicrobial compounds are bactericidal or bacteriostatic **(Bassole et al., 2001)**.

RESULTANTS

Chemical analysis

The yield of essential oil obtained by distillation of fresh material ranged from 0,1-0,2% (mL per 200 g of fresh material). Twenty-eight volatile components of essential oil of *C. ladanifer*, were identified and quantified. Figure 1 shows the chromatogram of *C. ladanifer* oil on a DB-5 column, while Table 1 lists Kováts retention indices and percentage composition of the compounds which have been identified in this oil. A total of 28 compounds, amounting to 88.67% of the oil, were identified. Mono- and sesquiterpene hydrocarbons represented the most important fraction of the total oil composition (44.45%), while of the six monoterpene hydrocarbons identified (32.05%), the major ones were Verticiol (18.16%), camphene (17.70%), n-Butylcyclohexane (5.95%) and 3-Carene (5.23%). The 6 sesquiterpene hydrocarbons were relatively low (12.4%) with γ-Gurjunene (7.15%) being the major component. The oxygenated mono- and sesquiterpenoids made up an appreciable fraction of the oil (15.84%). Isolongifolol was the major component of the Oxygenated sesquiterpenes (1.41%), while of the 9 oxygenated monoterpenes identified (13.27%), the most important ones were *cis*-Sabinol (5.14%), *trans*-Sabinene hydrate (1.85%) and 1-p-Menthen-9-al (1.52%).

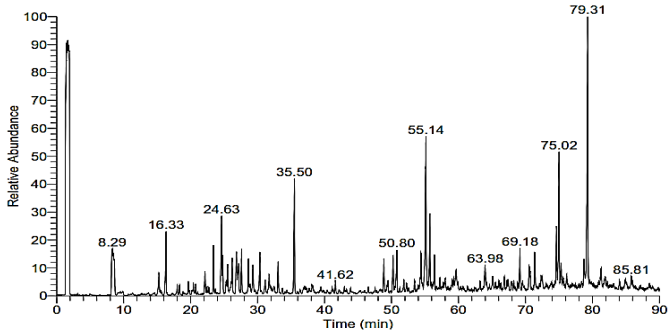

Figure 1 Chromatogram of *C. ladanifer* oil on a DB-5 column.

Table 1 Chemical composition of *Cistus ladanifer* var. *maculatus* Dun essential oil

KI	Compounds	Formula	Percentage
952	Camphene	$C_{10}H_{16}$	17,70
978	n-Butylcyclohexane	$C_{10}H_{20}$	5,95
1011	3-Carene	$C_{10}H_{16}$	5,23
1125	α-Campholenal	$C_{10}H_{16}O$	1,45
1026	α-Pinene	$C_{10}H_{16}$	1,03
1041	o-Cymene	$C_{10}H_{14}$	1,26
1090	o-Cymenene	$C_{10}H_{12}$	0,88
1097	*trans*-Sabinene hydrate	$C_{10}H_{18}O$	1,85
1143	*cis*-Sabinol	$C_{10}H_{16}O$	5,14
1166	Borneol	$C_{10}H_{18}O$	0,42
1193	Myrtenal	$C_{10}H_{14}O$	1,22
1194	Myrtenol	$C_{10}H_{16}O$	1,44
1205	*cis*-Verbenone	$C_{10}H_{14}O$	0,11
1205	Verbenone	$C_{10}H_{14}O$	0,12
1217	1-p-Menthen-9-al	$C_{10}H_{16}O$	1,52
1285	Bornyl acetate	$C_{12}H_{20}O_2$	5,86
1440	α-Guaiene	$C_{15}H_{24}$	0,35
1473	γ-Gurjunene	$C_{15}H_{24}$	7,15
1505	α-Bulnesene	$C_{15}H_{24}$	0,12
1537	β-Cadinene	$C_{15}H_{24}$	3,79
1542	α-Calacorene	$C_{15}H_{20}$	0,24
1581	Caryophyllene oxide	$C_{15}H_{24}O$	0,26
1617	Sclareol oxide	$C_{18}H_{30}O$	4,36
1642	Cubenol	$C_{15}H_{26}O$	0,65
1723	Isolongifolol	$C_{15}H_{26}O$	1,41
1772	Guaiazulene	$C_{15}H_{18}$	0,75
1890	Ledene oxide	$C_{15}H_{24}O$	0,25
2273	Verticiol	$C_{20}H_{34}O$	18,16
Total			**88,67**
Monoterpene Hydrocarbons			32,05
Sesquiterpene hydrocarbons			12,4
Oxygenated hydrocarbons			13,27
Oxygenated sesquiterpenes			2,57
Monoterpenic ester			5,86
Cyclic ether			4,36
Diterpenic alcohol			18,16

KI: Kováts indices, MS: NIST 98 spectra and the literature (Adams 2007), ST: Co injection with authentic standards% (v/v)

Antimicrobial activity

Diffusion method

In this work, antimicrobial activity of essential oil of *C. ladanifer* against four bacterial strains and three fungal strains, known to cause several infections in humans, was evaluated *in vitro*. The bacteria tested E.c, P.a, S.e show a zone of inhibition lower than that detected by the antibiogram CFM **(Ben Sassi et al., 2007; Mohammedi and Atik, 2011)**.These strains are resistant to gentamycin, but S.a is sensitive to it since it has a zone of inhibition of 23 mm which is greater than 20 mm.

The results of the sensitivity test, of microbial strains to essential oil of *C. ladanifer* by the aromatogramme method (Table 2), show inhibition of bacterial growth proportional to its concentration, which is related to the diameter of the zone of inhibition.

Table 2 Inhibition zones caused by the essential oil in mm of *C. ladanifer* at different concentrations.

	Bacteria tested (mm)				**Fungi tested (mm)**		
Concentrations of essential oil (µL)	*E.c*	*P.a*	*S.e*	*S.a*	*C.a*	*T.r*	*A.n*
5	9	NA	9	14	22	18	14
10	9	9	10	20	32	27	20
20	11	9	13	23	38	28	23
20 (RA/RF)	13	12	15	23	36	31	23

E.c (*Escherichia coli*), P.a (*Pseudomonas aeruginosa*), S.e (*Staphylococcus epidermidis*), S.a (*Staphylococcus aureus*), A.n (*Aspergillus niger*), T.r (*Trichophyton rubrum*), C.a (*Candida albicans*), RA (Antibiotic reference: Gentamicin), RF (antifungal Reference: Fluconazole) NA (no active).

Analyzing the results of the aromatogram of the essential oil of this plant, we note that all the diameters of inhibition are greater or equal to 9 mm for all the concentrations used and for all the bacteria tested. These results are in favor of an antibacterial capacity of this plant, by referring to the reading established by

Meena and Sethi, (1994) and **Ponce et al. (2003)**. The diameter of the inhibition halos is noted as: not sensitive for diameters less than 8 mm; sensitive, for diameters 9-14 mm; very sensitive for diameters 15-19 mm, and extremely sensitive for diameters larger than 20 mm. Thus, *Staphylococcus aureus* shows extreme sensitivity, and the three other bacteria respond very positively to its antibacterial effect, since they are classed in Gram positive or Gram-negative bacteria, and they are already have innate or acquired resistance to antibiotics. It should be noted that these bacteria selected in this study are the most incriminated in the vast majority of human infectious diseases, nosocomial infections and food poisoning. The continuous evolution of their resistance is one of the main causes of anti-biotherapy failure. Their eradication by the essential oil of *C. ladanifer* is a therapeutic success and is in fact a possible opportunity to disappoint treatment to antibiotics.

These results are consistent with those found in literature, some studies have found significant antibacterial activity of *C. ladanifer* on strains of *S. epidermidis* and *S. aureus* than on Gram negative strains like *E. coli* and *P. aeruginosa* which are among the most difficult germs to treat clinically **(Friedman et *al*., 2002; Burt, 2004)**. The intrinsic resistance to antibiotics makes them little vulnerable to the action of most antibacterial agents of natural or synthetic origin **(Lomovskaya et al., 2007)**.

This study also shows that all doses essential oil of *C. ladanifer* showed an inhibitory capacity of mycelial growth as well as spore production. The diameter of zone of inhibition vary between 38 mm in *C. albicans* and 28 mm in *T. rubrum*, which is greater than the values of reference used here. Our results on fungal strains are in agreement with the work of **Barros et al., (2013)** and **Greche et al. (2009)**, who showed a strong antifungal activity of *C. ladanifer* on some fungal strains.

Microdilution method

The minimum inhibitory concentration (MIC) values revealed that this oil was effectiveness against the tested bacteria and fungi and confirmed that it has broad spectrum antimicrobial effect.

The antibacterial effect unveiled seems to be strain dependent. In fact, strong inhibitory effect has been shown against all Gram-positive bacteria, especially *S. epidermidis* and *S. aureus*, since they were inhibited by very low concentrations 6.25 and 12.5 mg/mL (v/v) respectively. *E. coli* and *P. aeruginosa* are also sensible, but the MIC value is slightly greater 25 mg/mL (v/v). Previous studies confirmed that this latter bacterial strain was the most resistant **(Ghannadi et al., 2012; Boukhatem et al., 2013; Haloui et al., 2015)**. Furthermore, concerning the antifungal activity against molds and yeasts (Table3), the screening test revealed that all tested fungal strains were sensitive to the *C. ladanifer* essential oil. Indeed, the strongest inhibitory effect was exerted against yeasts and molds (*C. albicans* and *A. niger*) with MIC values of 0.001 mg/mL (v/v). These results are in agreement with those of previous works **(Hassane et al., 2011; Boukhatem et al., 2013)**. As regards to the fungal (*T. rubrum*) they were inhibited with MIC value of 0,01 mg/mL (v/v). The broad spectrum and the significant antimicrobial activity of the tested essential oil may be attributed to the synergistic interaction between the various components of this oil. In fact, it has been reported in previous studies that the inhibitory activity of an essential oil results from a complex interaction between its different constituents **(Burt, 2004; Xianfei et al., 2007; Viuda-Martos et al., 2008)**.

Essential oils, extracted from plants of the genus *Cistus* sp, are known for their complex chemical composition and diversity of their constituents. They contain a large number of molecules, often present in trace and very close chemical polarity. These two characteristics complicate the separation and identification of these same molecules **(Weyersthal et al., 1998)**.

Also, the share of unidentified compounds in the essential oils of *Cistus* sp, is generally not negligible. The chemical composition of the essential oil of *C. ladanifer* is chemotype Verticiol (18.16%), Camphene (17.70%), γ-Gurjunene (7.15%), n-Butylcyclohexane (5.95%) and Bornyl acetate (5.86%). These constituents are common to most essential oils of *C. Ladanifer* **(Mariotti et al., 1997)**. Oxygen molecules, which are part of the composition of essential oils, are generally more active than hydrocarbon molecules, known for their poor antibacterial properties **(Kalemba and Kunicka, 2003)**.

The hydrocarbon terpenes of the essential oil of *C. ladanifer* are also inactive. In contrast, oxygenated molecules inhibit the growth of most bacterial strains. The activity of the oxygenated molecules of essential oils depends both on the lipophilic nature of their hydrocarbon backbone and the hydrophilic nature of their functional groups **(Kalemba and Kunicka, 2003)**. Molecules belonging to different chemical classes will therefore not produce the same effects according to the bacterial strains tested. Phenols are, because of the acidity of their hydroxyl substituent, described as the most active compounds.

Carbonyl compounds, functionalized with aldehyde or ketone groups, and alcohols are also recognized for their antibacterial properties **(Kalemba and Kunicka, 2003)**. These two classes of molecules are part of the chemical composition of the essential oil of *C. ladanifer*. However, only the class of terpene alcohols has proved to be active. Gram negative bacteria are also very sensitive to the action of this class of molecules.

Bouamama et al. (2006) reported MIC values of crude extracts of *C. villosus* and *C. monspeliensis* against *S. aureus* of 0.76 and 25 mg/mL, respectively, and of 3.125 to 50 mg/mL for *P. aeruginosa*. Considering the antimicrobial activity of *C. ladanifer*, our work showed better results than *C. monspeliensis* against *S. aureus* and then the above both *Cistus* species against *P. aeruginosa*.

In this study, the minimal bactericidal concentration (CMB) and the minimum fungicidal concentration (CMF) were evaluated for the essential oil of *C. ladanifer*. The CMB/MIC and CMF / MIC ratios of *C. ladanifer* oil are equal to one for all microbial strains studied. This essential oil therefore seems to have a bactericidal and fungicidal action against all the bacterial and fungal strains tested. Table 3 shows the CMB and CMF of *C. ladanifer* essential oil against the tested germs.

Table 3 MIC, MBC and MFC values of essential oil of *C. ladanifer*.

Microbial strains	MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)	MBC/MIC MFC/MIC
E. coli	25	25	-	1
P. aeruginosa	25	25	-	1
S. epidermidis	12,5	12,5	-	1
S. aureus	6,25	6,25	-	1
A. niger	0,001	-	0,001	1
T. rubrum	0,01	-	0,01	1
C. albicans	0,001	-	0,001	1

CONCLUSION

The findings highlighted that *C. ladanifer* essential oil was able to inhibit the growth of a wide spectrum of microbial strains, known for their implications in human and animal infections. Its content on several antimicrobial active compounds and their likely interactions explained well its bioactivity. Hence, this essential oil can be a promising agent to control microbial growth, even if more detailed reports on its toxicity and mechanisms of action are requested to overcome the impediment of its application in several industries.

Acknowledgement : My thanks go to Colonel Pr. Lhoussain LOUZI, Head of Department of the Microbiology Laboratory, Military Hospital Moulay Ismail, Meknes (Morocco) for providing the fungal strains.

REFERENCES

Adams, R.P. (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Carol Stream, IL: Allured Publishing Corporation, Carol Stream, IL, USA 456. https://doi.org/10.1016/s1044-0305(97)00026-3

Anastassaki, T., Demetzos, C., Perdetzoglou, D., Gazouli, M., Loukis, A., & Harvala, C. (1999). Analysis of lab-dane-type diterpenes from *Cistus creticus* (subsp. creticus and subsp. eriocephalus), by GC and GC-MS. *Planta Med*, *65*, 735-739. https://doi.org/10.1055/s-1999-14095

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils-a review. *Food Chem Toxicol*, *46*, 446-475. https://doi.org/10.1016/j.fct.2007.09.106

Bassole, H.N., Kabore, Z.I., & Traore, A.S. (2001). Étude des profils bactériostatiques et bactéricides d'extraits végétaux vis-à-vis de germes pathogènes impliqués dans la contamination des denrées alimentaires d'origines animale. *Pharm Méd Trad Afr*, *11*, 113-122. http://greenstone.lecames.org/collect/revueph1/index/assoc/HASHd7b6.dir/11-113-122.pdf

Ben Sassi, A., Harzallah-Skhiri, F., & Aouni, M. (2007). Investigation of Some Medicinal Plants from Tunisia for Antimicrobial Activities. *Pharmaceutical Biology*, *45*(5), 421-428. https://doi.org/10.1080/13880200701215406

Bouamama, H., Noel, T., Villard, J., Benharref, A., & Jana, M. (2006). Antimicrobial activities of the leaf extracts of two Moroccan *Cistus* L. species. *Journal of Ethnopharmacology*, *104*(1), 104-107. https://doi.org/10.1016/j.jep.2005.08.062

Barrajón-Catalán, E., Fernández-Arroyo, S., Roldán. C., Guillén, E., Saura, D., Segura Carretero, A., Micol, V. (2011). A systematic study of the polyphenolic composition of aqueous extracts deriving from several *Cistus* genus species: Evolutionary relationship. Phytochem. Anal, 22, 303-312. https://doi.org/10.1002/pca.1281

Barrajón-Catalán, E., Fernández-Arroyo, S., Saura, D., Guillén, E., Fernández-Gutiérrez, A., Segura-Carretero, A., Micol, V. (2010). Cistaceae aquous extracts containing ellagitannins show antioxidant and antimicrobial capacity, and cytotoxic activity against human cancer cells. *Food Chem. Toxicol*, *48*, 2273-2282. https://doi.org/10.1016/j.fct.2010.05.060

Barros, L., Dueñas, M., Alves, C.T., Silva, S., Henriques, M., Santos-Buelga, C. (2013). Antifungal activity and detailed chemical characterization of *Cistus ladanifer* phenolic extracts. *Ind. Crops Prod*, *41*, 41-45. https://doi.org/10.1016/j.indcrop.2012.03.038

Benayad, N., Mennane, Z., Charof, R., Hakiki, A., Mosaddak, M. (2013). Antibacterial activity of essential oil and some extracts of *Cistus ladaniferus* from Oulmes in Morocco. *J. Mater. Environ. Sci*, *4* (6), 1066-1071. https://www.jmaterenvironsci.com/Document/vol4/vol4_N6/143-JMES-443-2013-Benayad.pdf

Boudy, P., 1951. North African forest economy. Volume 3rd Forest description of Morocco. F. Moncho Editions Rabat 79.

Boukhatem, M.N., Kameli, A., & Saidi, F. (2013). Essential oil of Algerian rose-scented geranium (*Pelargonium graveolens*): Chemical composition and antimicrobial activity against food spoilage pathogens. *Food Control*, *34*, 208-213. https://doi.org/10.1016/j.foodcont.2013.03.045

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of food Microbiology*, *94*, 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Chinou, I., Demetzos, C., Harvala, C., Roussakis, C., & Verbist, J.F. (1994). Cytotoxic and antibacterial labdane-type diterpenes from the aerial parts of *Cistus incanus* subsp. creticus. *Planta Med*, *60*, 34-36. https://doi.org/10.1055/s-2006-959403

Chraibi, M., Farah, A., Balouiri, M., Barkai, H., Sadiki, M., & Benbrahim, K.F. (2016). Chemical characterization and antimicrobial activity of Moroccan *Pelargonium asperum* essential oil. *Journal of Applied Pharmaceutical Science*, *6*(12), 042-046. https://doi.org/10.7324/japs.2016.601206

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*, 564-582. https://cmr.asm.org/content/cmr/12/4/564.full.pdf

Cushnie, T.P.T., & Lamb, A.J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *Int J Antimicrob Agents*, *38*, 99-107. https://doi.org/10.1016/j.ijantimicag.2011.02.014

Daouk, R.K., Dagher, S.M., & Sattout, E.J. (1995). Antifungal Activity of the Essential Oil of Origanum syriacum L. *J Food Prot*, *58*, 1147-1149. https://doi.org/10.4315/0362-028x-58.10.1147

Delgado, J.A., Serrano, J.M., López, F., Acosta, F.J. (2001). Heat shock, massdependent germination, and seed yield as related components of fitness in *Cistus ladanifer*. *Environ Exp Bot* ,*46*(1), 11-20. https://doi.org/10.1016/s0098-8472(01)00076-4

Demetzos, C., Harvala, C., Philianos, S.M., & Skaltsounis, A.L. (1990). A new labdane-type diterpene and other compounds from the leaves of *Cistus incanus* subsp *creticus*. *J Nat Prod*, *53*, 1365-1368. https://doi.org/10.1021/np50071a039

Demetzos, C., Loukis, A., Spiliotis, V., Zoakis, N., Stratigakis, N., & Katerinopoulos, H. (1994a). Composition and antimicrobial activity of the essential oil of *Cistus creticus* L. *J Ess Oil Res*, *7*, 407-410. https://doi.org/10.1080/10412905.1995.9698549

Demetzos, C., Mitaku, S., Couladis, M., Harvala, C., & Kokkinopoulos, D. (1994b). Natural metabolites of ent-13-epi-manoyl oxide and other cytotoxic diterpenes from the resin ladano of *Cistus creticus*. *Planta Med*, *60*, 590-591. https://doi.org/10.1055/s-2006-959584

Demetzos, C., Mitaku, S., Skaltsounis, A.L., Couladis, M., Harvala, C., & Libot, F. (1994c). Diterpene esters of malonic acid from the resin ladano of *Cistus creticus*. *Phytochemistry*, *35*, 979-981. https://doi.org/10.1016/s0031-9422(00)90651-4

Dimas, K., Demetzos, C., Marsellos, M., Sotiriadou, R., Malamas, M., & Kokkinopoulos, D. (1998). Cytotoxic activity of labdane type diterpenes against human leukemic cell lines *in vitro*. *Planta Med*, *64*, 208-211. https://doi.org/10.1055/s-2006-957410

Eloff, J.N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, *64*, 711-713. https://doi.org/10.1055/s-2006-957563

Fauchère, J.L., & Avril, J.L. (2002). Bactériologie générale et médicale. Ellipses *Editions Paris* 365. https://www.unitheque.com/Livre/ellipses/Bacteriologie_generale_et_medicale-1724.html

Fernández-Arroyo, S., Micol, V., Segura-Carretero, A., Fernández-Gutiérrez, A., Barrajón-Catalán, E. (2010). High-performance liquid chromatography with diode array detection coupled to electrospray time-of-light and ion-trap tandem mass spectrometry to identify phenolic compounds from a *Cistus ladanifer* aqueous extract. *Phytochem. Anal*, *21*, 307-313. https://doi.org/10.1002/pca.1200

Ferreira, S., Santos, J., Duarte, A., Duarte, A.P., Queiroz, J.A. and Domingues, F.C. (2012). Screening of antimicrobial activity of *Cistus ladanifer* and *Arbutus unedo* extracts, *Natural Product Research*: Formerly Natural Product Letters, 26:16, 1558-1560. https://doi.org/10.1080/14786419.2011.569504

Friedman et al. (2002). Bactericidal Activities of Plant Essential Oils and Some of Their Isolated Constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and Salmonella enteric. *Journal of Food Protection*, *65*(10), 1545-1560. https://doi.org/10.4315/0362-028x-65.10.1545

Garcia-Martin, D., Garcia-Vallejo, C. (1969). Contribution à la connaissance de l'huile essentielle de *Cistus ladanifer* var *maculatus* Dun (Ciste commun-jara d'Espagne). Parfumerie Cosmétique et Savon, 12, 283-290.

Ghannadi, A., Bagherinejad, M.R., Abedi, D., Jalali, M., Absalan, B., & Sadeghi, N. (2012). Antibacterial activity and composition of essential oils from *Pelargonium graveolens* L. Her and Vitex agnus-castus L. *Iran J Microbiol*, *4*(4), 171-176. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3507305/

Greche, H., Mrabet, N., Zrira, S., Ismaïli-Alaoui, M., Benjilali, B., Boukir, A. (2009). The Volatiles of the Leaf Oil of *Cistus ladanifer* L. var. *albiflorus* and Labdanum Extracts of Moroccan Origin and their Antimicrobial Activities. *Journal of Essential Oil Research*, *21*(2), 166-173. https://doi.org/10.1080/10412905.2009.9700140

Gültz, P.G., Herrmann, T., & Hangst, K. (1996). Leaf trichomes in the genus Cistus. *Flora*, *191*, 85-104. https://doi.org/10.1016/s0367-2530(17)30692-8

Gülz, P.G., Kobold, U., & Vostbowsky, O. (1984). The composition of terpene hydrocarbons in the essential oils from leaves of *Cistus* species. *Z Naturforsch*, *39c*, 699-704. https://doi.org/10.1515/znc-1984-7-802

Gülz, P.G., Proksch, P. (1984). Methylated flavonoids from *Cistus ladanifer* and *Cistus palhinhae* and their taxonomic implications. *Phytochemistry*, *23*, 470-471. https://doi.org/10.1016/s0031-9422(00)80359-3

Haloui, T., Farah, A., Balouiri, M., Chraibi, M., Fadil, M., Benbrahim, K., & Alaoui, A. (2015). Bacteriostatic and Bactericidal Profile of Leaves and Twigs Essential oils of Moroccan *Pistacia lentiscus* L. *J Appl Pharm* Sci, *5*, 50-53. https://doi.org/10.7324/japs.2015.50607

Hassane, S.O.S., Ghanmi, M., Satrani, B., Mansouri, N., Mohamed, H., El Hajaji, H., & Chaouch, A. (2011). Composition chimique et activités antibactériennes, antifongiques et antioxydante de l'huile essentielle de *Pelargonium asperum* Ehrh. ex Wilde des Comores. *Acta Bot Gall*, *158*, 225-237. https://doi.org/10.1080/12538078.2011.10516269

Kalemba, D. & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Curr Med Chem*, *10*, 813-829. https://doi.org/10.2174/0929867033457719

Kasrati, A., Alaoui, J.C., Fadli, M., Bekkouche, K., Hassani, L., & Wohlmuth, H. (2014). Antioxidative activity and synergistic effect of *Thymus saturejoides* Coss. essential oils with cefixime against selected food-borne bacteria. *Industrial Crops and Products*, *61*, 338-344. https://doi.org/10.1016/j.indcrop.2014.07.024

Konigs, R., Gülz, P.G. (1974). The monoterpenes in the essential leaf oil of *Cistus ladaniferus* L. *Z.P flanzenphysio*, *72*, 237-248. https://doi.org/10.1016/s0044-328x(74)80053-x

Lomovskaya, O., Zgurskaya, H.I., Totrov, M., Watkins, & W.J. (2007). Waltzing transporters and 'the dance macabre' between humans and bacteria. *Nat. Rev Drug Discov*, *6*, 56-65. https://doi.org/10.1038/nrd2200

Mahesh, B. & Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J Agri Sci*, *4*(S), 839-843. https://www.idosi.org/wjas/wjas4(s)/7.pdf

Mariotti, J.P., Tomi, F., & Bernardini, A.F. (1994). Étude d'huiles essentielles de *Cistus ladaniferus* L. cultivé en Corse. *Rivista Italiana EPPOS*, 614-620. https://doi.org/10.1002/(sici)10991026(199705)12:3%3c147::aidffj631%3e3.0.co;2-q

Mariotti, J.P., Tomi, F., Casanova, J., Costa, J., & Bernardini, A.F. (1997). Composition of the essential oil of *Cistus ladaniferus* L. cultivated in Corsica (France). *Flavour Fragr J*, *12*, 147-151. https://doi.org/10.1002/(sici)1099-1026(199705)12:3%3C147::aid-ffj631%3E3.3.co;2-h

Meena, M.R. & Sethi, V. (1994). Antimicrobial activity of the essential oils from spices. *Food Science and Technology*, *31*, 68-70.

Mohammedi, Z. & Atik, F. (2011). Antibacterial activity of essential oils from *Cistus ladaniferus* L. and *Lavandula stoechas* L. *International Journal of PharmTech Research*, *3*(1), 484-487. https://pdfs.semanticscholar.org/71e5/5bce5da35a6c4980ad5b536058fddf89604b.pdf

Mrabet, N., Lahlou, H., Benjilali, B. (1999). Effect of Moroccan *C. ladaniferus* L. (rockrose) extracts on the growth of four fungi. *Cryptogam. Mycol*, *20*, 23-33. https://doi.org/10.1016/s0181-1584(99)80005-0

Nsambu, M., Muhigwa, B., Rubabura, K., Bagalwa, M., & Bashwira, S. (2014). *In vitro* evaluation of the insecticidal activity of alkaloids, saponins, terpenoids and steroids extracts *Capscicum frutescens* L. (Solanaceae) against Antestiopsis orbitalis ghesquierei, pest insects of cafeirs. *Int J Innov Appl Stud*, *8*, 1231-1243. file:///C:/Users/MedSim/Downloads/IJIAS-14-236-06.pdf

Ponce, A.G., Fritz, R., del Valle, C., & Roura, S.I. (2003). Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *Lebensm-Wiss-senschaft und-Technol*, *36*, 679-684. https://doi.org/10.1016/s0023-6438(03)00088-4

Peyron, L., Alessandri, A. (1986). Huile essentielle de feuillage de *Cistus ladaniferus* L. cultivé en Corse. Parfums, Cosmétiques, Arômes, 67, 59-67.

Prasannabalaji, K.N., Muralitharan, G., Sivanandan, R.N., Kumaran, S., & Pugazhvendan, S.R. (2012). Antibacterial activities of some Indian traditional plant extracts. *Asian Pac J of Trop Dis*, 291-295. https://doi.org/10.1016/s2222-1808(12)60168-6

Proksch, P., Gülz, P.G., Budzikiewicz, H. (1980). Further oxygenated compounds in the essential oil of *Cistus ladanifer* L (Cistaceae). *Z Naturforsch, 35c*, 529-532. https://doi.org/10.1515/znc-1980-7-801

Ramalho, P.S., Victor, A.P., Freitas, A., Macedo, G., Silva and Silva A.M.S. (1999). Volatile components of *Cistus ladanifer* leaves. *Flav. Fragr. J.*, *14*, 300-302. https://doi.org/10.1002/(sici)1099-1026(199909/10)14:5%3C300::aid-ffj830%3E3.0.co;2-x

Robles, C., Bousquet-Mélou, A., Garzino, S., Bonin, G., (2003). Comparison of essential oil composition of two varieties of *Cistus ladanifer*. Biochem. *Syst. Ecol*, *31*, 339-343. https://doi.org/10.1016/s0305-1978(02)00161-8

Salah-Fatnassi, K.B.H., Slim-Bannour, A., Harzallah-Skhiri, F., Mahjoub, M.A., Mighri, Z., Chaumont, J.P., & Aouni, M. (2010). Activités antivirale et antioxydante *in vitro* d'huiles essentielles de *Thymus capitatus* (L.) Hoffmans. & Link de Tunisie. *Acta Bot Gall*, *157*, 433-444. https://doi.org/10.1080/12538078.2010.10516220

Simon-Fuentens, A., Sendra, J.M., & Cunat, P. (1987). Neutral Volatiles of *Cistus ladaniferus* L. Essential Oil. *An Quim*, *83*(C), 201-204. http://www.worldcat.org/title/environmental-chemistry-fundamentals/oclc/1002947336

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez Álvarez, J.A. (2008). Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet. *Int J Food Sci Technol*, *43*, 526-531. https://doi.org/10.1111/j.1365-2621.2006.01489.x

Vogt, T., Proksch, P., & Gültz, P.G. (1987b). Rare 6 and 8-O methylated epicuticular flavonoids from two *Cistus* species. *Phytochemistry*, *26*, 1027-1030. https://doi.org/10.1016/s0031-9422(00)82342-0

Vogt, T., Proksch, P., & Gültz, P.G. (1987a). Epicuticular flavonoids in the genus *Cistus*, Cistaceae. *J Plant Phy siol*, *131*, 25-36. https://doi.org/10.1016/s0176-1617(87)80264-x

Weyerstahl, P., Marschall, H., Weirauch, M., & Thefeld, K. (1998). Surburg H. Constituents of commercial Labdanum oil. *Flavour Fragr* J, *13*, 295-318. https://doi.org/10.1002/(sici)1099-1026(1998090)13:5%3C295::aid-ffj751%3E3.0.co;2-i

Xianfei, X., Xiaoqiang, C., Shunying, Z., & Guolin, Z. (2007). Chemical composition and antimicrobial activity of essential oils of *Chaenomeles speciosa* from China. *Food Chem*, *100*, 1312-1315. https://doi.org/10.1016/j.foodchem.2005.12.011