Essential Oil from Cistus ladanifer L.

Subjects: Microbiology

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In Oulmes (Middle Atlas, Morocco), the local population uses C. ladanifer traditionally to treat various diseases and health issues due to its antioxidant, gastric, anti-inflammatory, antitumor, antimicrobial, and antiviral properties. This plant is usually harvested in May in this area (flowering time). The leaves of all Cistus species secrete essential oils. Essential oils from different species of medicinal plants have been documented to possess antimicrobial propriety with strong activity against Gram-negative and Gram-positive bacteria and also fungi.

C. ladanifer var. maculatus Dun | essential oil | antimicrobial activity | yeast | mold | chemical analysis

1. Introduction

Aromatic and medicinal plants (AMPs) have long been a part of man's everyday existence for a variety of purposes. AMPs play a crucial and fundamental role in traditional medicine and play a very significant role in drug discovery [1]. Natural substances account for around 25% of all medicines available for the treatment of illnesses (plants, animals, bacteria, and fungi) [2]. *Cistus ladanifer* L. is one of the medicinal plants, belonging to the Cistaceae family, the latter represented by seven genera (*Cistus, Fumane, Halimium, Tuberaria, Helianthemum, Hudsonia* and *Lechea*), the genus *Cistus* alone encompass 16 species particularly distributed in the Mediterranean region [3][4], this kind is widespread in Portugal, Spain, Italy, Algeria, and Morocco [5]. The most common species are *C. ladanifer* (Gum Cistus), *C. monspeliensis* (Montpellier Cistus), *C. salviifolius* (Sage Cistus), *C. laurifolius* (Laurel Cistus), *C. creticus* (Cretan Cistus), and *C. albidus* (Cottony Cistus). *C. ladanifer* is represented in Morocco by two varieties that differ mainly by the color of the petals of flowers: *C. ladanifer* var. *albiflorus* Dun with completely white petals and *C. ladanifer* var. *maculatus* Dun with petals spotted with crimson. *C. ladanifer* is a much-exploited plant in Morocco, it is known as 'Touzzalt' in Amazigh, and in Arabic, it is called kastousse, Bouzegzaw, ftah, Targla, Touzzala' [6]. It is a very fragrant spontaneous shrub; it has sticky branches up to 2 m tall, large white flowers (64 mm in diameter) that appear during spring (March–May) and have a three-day lifespan. Its seeds appear between July and October [7]. This species grows in very diverse climates; it is extremely resistant to cold stress, drought, and high temperatures [8].

In Oulmes (Middle Atlas, Morocco), the local population uses *C. ladanifer* traditionally to treat various diseases and health issues due to its antioxidant, gastric, anti-inflammatory, antitumor, antimicrobial, and antiviral properties [9][10][11]. This plant is usually harvested in May in this area (flowering time) [12]. The leaves of all *Cistus* species secrete

essential oils [13]. Essential oils from different species of medicinal plants have been documented to possess antimicrobial propriety with strong activity against Gram-negative and Gram-positive bacteria and also fungi [14].

C. ladanifer essential oil is characterized by a large number of sesquiterpenes (viridiflorol and ledol) and monoterpenoids (bornyl acetate and pinocarveol) [15] that are maybe behind several reported activities such as analgesic, anti-inflammatory [16], antiplatelet [17], antioxidant [10][18], antidiarrheal, antispasmodic, anti-acid [19][20], antiulcer [21], antitumor and gastroprotective activities [22]. Sosa et al. confirmed that aqueous *Cistus* extract could be considered an inhibitor of calcium transport in skeletal muscles [23]. Belmoukhtar et al. and Aziz et al. showed that aqueous extract of *C. Ladanifer* has also been considered as a curative and preventive treatment for hypertension and the possibility of using it in the treatment of gastrointestinal disorders [20][24]. Amensour et al. reported that *C. ladanifer* is a source of natural antioxidants that can be exploited in the food industry given the high levels of existing flavonoids and phenolic compounds [25]. Finally, Andrade et al. and Barrajón-Catalánet al. have also reported its cytotoxic potential against several human cancer cells [10][11]. Due to bacterial and fungal resistance and the side effects of antibiotics, great interest has been given to biologically active molecules isolated from plant species [26], in particular, essential oils [19]. The objective of this work is to study for the first time the effect of *C. ladanifer* essential oil at its flourishing time (April) and the collection region (Oulmès region, Middle Atlas) on the chemical composition and antimicrobial effect of *C. ladanifer* var. maculatus Dun.

2. Current Results

2.1. C. Ladanifer Moisture Content and Its Essential Oil Yield Percentage

The moisture content of *C. ladanifer* essential oil was 13.2%. The yield extraction of the essential oil was around 0.21 ± 0.01%. These results were quite high compared to those obtained by several other researchers. In France, Robles reported a yield of 0.119 ± 0.016% [27]. In Algeria, Bechlaghem reported a yield of 0.08% [28]. Zidane et al. from Morocco obtained a yield of 0.14% [29]. Grech et al., also from Morocco found a yield of 0.3 to 0.4% in the northern region [30]. Thus, we find that the *C. ladanifer* essential oil yield, although highly variable, remains relatively low regardless of the region and the time of harvest.

2.2. Mineral (ash) and Organic Matter Content in the C. Ladanifer Essential Oil

The mineral content obtained was 3.7%, which was considered quite important. The organic content was around 1.35%. This variation can be explained by the mineral reserves of the soil, the efficiency of their root capture, and their movement towards the aerial organs of the *C. ladanifer*.

2.3. Refractive Index and Brix Index of the C. Ladanifer Essential Oil

The refractive index and Brix index are qualitative identification characteristics that may be used to evaluate the purity of essential oils [31]. Each substance has its specific refractive index. The purity of a product is determined by how near its refractive index is to the anticipated value. The refractive index of our studied essential oil is 1.45. Mrabet et al. reported a refractive index of 1.49 for the essential oil of *C. ladanifer* var maculatus from northern

Morocco [32]. The refractive index values of *C. ladanifer* essential oil extracted by hydrodistillation are comparable to those of standards, indicating that our extracts are of excellent purity confirmed also with the low Brix index (1.33) which is an indicator of the concentration (%) of all solids dissolved in the essential oil (**Table 1**).

Table 1. Refractive index and Brix index of the *C. ladanifer* essential oil.

Plant	Refractive Index	Brix Index
C. ladanifer L. essential oil	1.45	1.33556

2.4. GC-SM Analysis of the Essential Oil of C. Ladanifer

The GC-MS analysis revealed the presence of 35 compounds in the essential oil of *C. ladanifer* (**Figure 1**). These compounds were divided into oxygenated sesquiterpenes (34.02%), oxygenated monoterpenes (33.14%), linear esters (10.38%), monoterpenes (9.11%), and sesquiterpenes (4.29%). The major constituents present in this EO were viridiflorol (17.64%), *trans*-pinocarveol (11.02%), bornylacetate (9.38%), and ledol (8.85%) (**Table 2**). The percentage of these constituents is higher than those found by Boukil et al. (oxygenated hydrocarbons (13.27%), oxygenated sesquiterpenes (2.57%), and monoterpenic ester (5.86%)) [19]. The same authors found that the main components from the fresh leaves of *C. ladanifer* were verticiol (18.16%), camphene (17.70%), *n*-butylcyclohexane (5.95%), and 3-carene (5.23%) [19]. These findings indicate that the time of harvest has a significant impact on the chemical composition obtained. The results of various chemical analysis studies on *C. ladanifer* essential oil carried out previously showed that the main constituents of *C. ladanifer* leaf essential oil from Northern Morocco were viridiflorol (19.6%), bornyl acetate (16.7%), and camphene (12.3%) [30]. Zidane et al. characterized *C. ladanifer* from Eastern Morocco and indicated the presence of camphene (15.5%), borneol (11.1%), 2,2,6-trimethylcyclohexanol (7.3%), 4-terpineol (6.3%), and α-pinene (4.2%) as the major compounds in the essential oil of this plant [29]. In Algeria, it was found that the main constituents of this oil were 5,7-di-*epi*-α-eudesmol (13.6%), borneol (12.5%), camphene (12.2%), δ-cadinene (7.6%), α-eudesmol (6.4%)%), 4-terpineol (5.7%) and α-pinene (4.2%) [28]. In France, Verdeguer et al. characterized the chemical composition of the oil extracted from the leaves and stems of *C. ladanifer* of Spanish origin but cultivated in Corsica by the presence of pinene (39%), viridiflorol (11.8%), ledol (3.3%) and bornyl acetate (3.1%) [33]. In Portugal, the chemical composition of *C. ladanifer* oil shows the presence of three sesquiterpenes alcohols, viridiflorol (13.6–17.4%), globulol (3.1–5.0%), and an unknown alcohol sesquiterpene (2.7–6.0%), as well as diterpene alcohol 15-*nor*-labdan-8-ol (1.7–5.2%) [34]. In Spain, the composition of the essential oil of *C. ladanifer* cultivated in central Spain, revealed its richness in oxygenated compounds, with *trans*-pinocarveol (20.00%), bornyl acetate (7.03%), and terpinen-4-ol (6.37%) as the main monoterpene compounds. Viridiflorol (13.59%) and ledol (4.36%) were the main constituents of the oxygenated sesquiterpene fraction. Large amounts of α-pinene (4.70%) were found in the hydrocarbon fractions. From this comparison, it seems that Moroccan *C. ladanifer* essential oil composition is closer to that of Corsica with Spanish origin. The chemical composition of *C. ladanifer* essential oil varies considerably depending on the source, plant material, and extraction method. As a result, based on the intended product during the exploitation of the species, a selection of organs, vegetative stage, and area proves to be extremely helpful in promoting the acquisition of very accurate chemotypes.

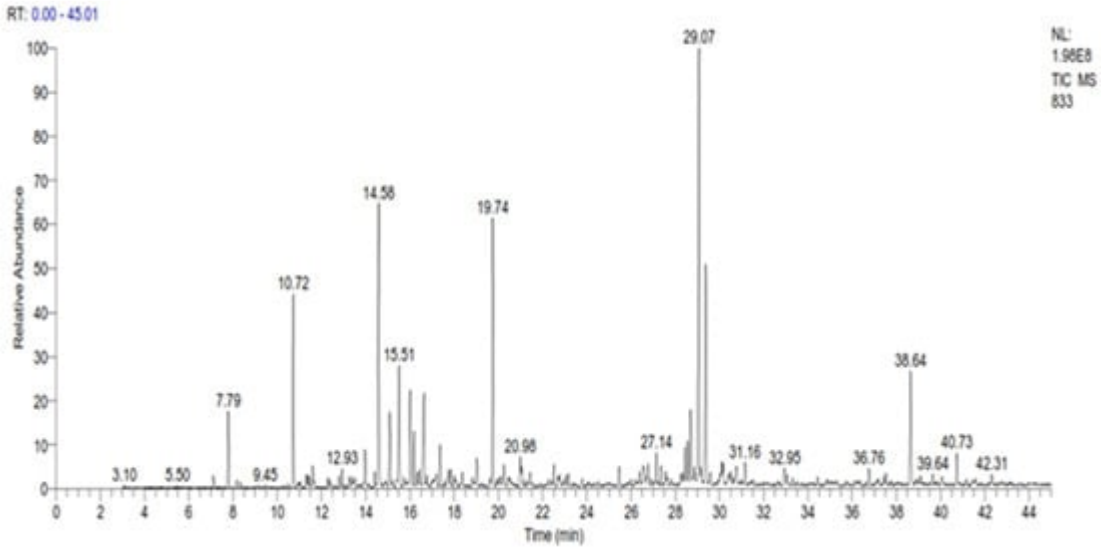

Figure 1. GC Chromatogram of the essential oil of *C. ladanifer*.

Table 2. The compounds identified in the essential oil of *C. ladanifer* after analysis by GC/MS on column DB-5.

No.	Compounds	Formulas	Percentage	IK Calculated	IK (ADAMS)
1	α-Pinene	$C_{10}H_{16}$	2.43	922	939
2	*p*-Cymene	$C_{10}H_{14}$	6.11	1004	1024
3	(*Z*)-vertocitral C	$C_9H_{14}O$	0.63	1028	1080
4	*p*-Cymenene	$C_{10}H_{14}$	0.57	1065	1091
5	α-Campholenal	$C_{10}H_{16}O$	1.34	1093	1126
6	*trans*-Pinocarveol	$C_{10}H_{16}O$	11.02	1110	1139
7	Pinocarvone	$C_{10}H_{14}O$	2.72	1125	1164
8	Borneol	$C_{10}H_{18}O$	4.80	1137	1169
9	Terpinen-4-ol	$C_{10}H_{18}O$	4.09	1151	1177
10	Myrtenal	$C_{10}H_{14}O$	1.76	1156	1195
11	Myrtenol	$C_{10}H_{16}O$	4.02	1168	1195
12	*trans*-Carveol	$C_{10}H_{16}O$	1.44	1189	1216
13	Carvone	$C_{10}H_{14}O$	0.54	1203	1243
14	(*Z*)-β-Damascone	$C_{13}H_{20}O$	0.97	1238	1387
15	Bornylacetate	$C_{12}H_{20}O_2$	9.38	1259	1285

No.	Compounds	Formulas	Percentage	IK Calculated	IK (ADAMS)
16	Carvacrol	$C_{10}H_{14}O$	0.80	1274	1084
17	Myrtenylacetate	$C_{12}H_{18}O_2$	1.00	1296	1326
18	2,4,6-trimethoxytoluene	$C_{10}H_{14}O_3$	0.61	1298	1483
19	(*E*)-Trimenal	$C_{13}H_{22}O$	0.77	1343	1421
20	Aromadendrene	$C_{15}H_{24}$	0.67	1438	1441
21	Viridiflorene	$C_{15}H_{24}$	0.95	1473	1496
22	2,3-Dihydro-1,1,4,5,6-pentamethyl 1*H*-indene	$C_{14}H_{20}$	0.81	1481	1522
23	*cis*-Calamenene	$C_{15}H_{22}$	1.17	1493	1529
24	δ-Cadinene	$C_{15}H_{24}$	0.68	1500	1523
25	Palustrol	$C_{15}H_{26}O$	1.14	1538	1568
26	Spathulenol	$C_{15}H_{24}O$	1.41	1542	1578
27	Caryophyllene oxide	$C_{15}H_{24}O$	2.85	1546	1583
28	Viridiflorol	$C_{15}H_{26}O$	17.74	1560	1592
29	Ledol	$C_{15}H_{26}O$	8.85	1570	1602
30	1,10-di-*epi*-Cubenol	$C_{15}H_{26}O$	0.75	1595	1619
31	Caryophylla-4 (12), 8 (13)-dien-5α-ol	$C_{15}H_{24}O$	0.64	1598	1640
32	Cadalene	$C_{15}H_{18}$	0.82	1634	1676
33	14-Hydroxy-4,5-dihydro-caryophyllene	$C_{15}H_{26}O$	0.64	1848	1706
34	Sclareol	$C_{20}H_{36}O_2$	4.60	1924	2223
35	13-*epi*-Dolabradiene	$C_{20}H_{32}$	1.28	2013	2000
	Oxygenated sesquiterpenes				34.02
	Oxygenated monoterpenes				33.14
	Linear esters				10.38
	Monoterpenes				9.11
	Sesquiterpenes				4.29

Antibacterial Activity

In this study, the antibacterial activity was evaluated using two methods: the agar disk diffusion method and the dilution in a liquid environment. The aim of the tests is to highlight the inhibitory power of the essential oil vis-à-vis

No.	Compounds	Formulas	Percentage	IK Calculated	IK (ADAMS)
	Others				9.06
	Total				100

The antibiotic sensitivity profiles of the strains are developed according to the recommendations of the Committee on Antibiotic susceptibility of the French Society for Microbiology (CA-SFM) and presented in **Table 3**. From the table, it can be concluded that the strains of *S. aureus* and *S. Typhi* were sensitive to all antibiotics, while *E. coli* was only resistant to ticarcillin. At the same time, *A. baumannii* demonstrated complete resistance to all tested antibiotics. This may be due to the higher resistance of Gram-negative bacteria due to the complexity of their cell wall, containing a double membrane in opposition to the single glycoprotein/teichoic acid membrane of Gram-positive bacteria [35]

Table 3. The test for the sensitivity of bacterial strains to certain antibiotics.

ATB	*A. baumannii*	ATB	*S. aureus*
TIC 75 µg	R	CIP 5 µg	S
CEF 30 µg	R	VAN 30 µg	S
MEM 10 µg	R	TET 30 µg	S
TIM 85 µg	R	CEF 15 µg	S
ATB	***E. coli***	**ATB**	***S. Typhi***
COL 50 µg	S	COL 50 µg	S
MEM 10 µg	S	MEM 10 µg	S
TIC 75 µg	R	TIC 75 µg	S
AMI 30 µg	S	AMI 30 µg	S

S: Sensitive; I: Intermediate; A: Resistant; ATB: Antibiotics; TIC: Ticarcillin; CEF: Ceftazidime; VAN: Vancomycin; TET: Tetracycline; CEF: Cefalexin; COL: Colistin; AMI: Amikacin.

2.5.2. The Agar Disk-Diffusion Method for *C. ladanifer* Essential Oil

Table 4 and **Figure 2** represent the results of the agar disk-diffusion method for *C. ladanifer* essential oil against the selected bacterial strains after 24 h at 37 °C. The agar disk diffusion method results indicated that the essential oil of *C. ladanifer* has a remarkable antibacterial activity compared to the concentration used (5 µL). From the analysis of the results obtained (**Table 4**), it was noted that the four microorganisms studied are sensitive except *S. Typhi* which demonstrated an inhibition zone of 30 ± 0.25 mm when the EO of *C. ladanifer* was used. The best inhibition diameter was against *S. aureus* (55 ± 0.22 mm) and *E. coli* (42 ± 0.11). It was also noted that contrary to the sensitivity test when *A. baumannii* demonstrated full resistance to the tested antibiotics, it demonstrated an inhibition diameter of 35 ± 0.27 when the EO was used, indicating a good and promising effect. Our results are superior to those found by Benayad et al. (*S. aureus* (28 mm), *A. Baumannii* (24 mm), and *E. coli* (18 mm)) (plant harvested in May) [11]. Same note for results obtained by Boukil et al. (*S. aureus* (14 mm), and *E. coli* (9 mm))

(plant harvested in August) [19]. As an outcome, harvesting the plant at the flowering stage is correlated to a potent antibacterial power.

Figure 2. Bacterial strain growth inhibition zone of *C. ladanifer* essential oil.

Table 4. Diameter of the inhibition zone of *C. ladanifer* essential oil against the four pathogenic strains.

	S. aureus	***E. coli***	***A. baumannii***	***S. Typhi***
C. ladanifer 5 µL (EO)	55 ± 0.22	42 ± 0.11	35 ± 0.27	30 ± 0.25

The inhibition diameters were expressed in millimeters (mm): Mean ± SD.

2.5.3. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)

During this investigation, the determination of the MIC was evaluated by assessing the inhibitory power of the plant's essential oil at different concentrations against the selected bacteria (**Table 5**). In total accordance with the previous results obtained in the agar disk diffusion method and the observed inhibition zone, the results obtained indicated that the *C. ladanifer* essential oil MIC and MBC for all strains were 10 µL/mL. Regarding the MBC/MIC activity ratio, our results indicate that it was equal to 1 for all strains. This value allows us to affirm that the essential oil of *C. ladanifer* is bactericidal. This antimicrobial activity of this essential oil can only be explained by its chemical profile rich in 34.02% of oxygenated sesquiterpenes and oxygenated monoterpenes, and 33.14% of monoterpenes which are known as versatile anti-infective agents (antibacterial and antifungal) [36]. The structures of the functional groupings of the constituents of essential oils could play a crucial role in determining the antibacterial power of essential oils [37]. Nevertheless, minority compounds can interact directly, or in a synergistic or in an antagonistic way, to create a mixture with biological activity. Guinoiseau et al. [38], Rossi et al. [39], and Vieira et al. [40] demonstrated that essential oil from *C. ladanifer* has antimicrobial activity against Gram-positive and negative pathogens of clinical importance such as *Staphylococcus aureus*, *E. coli*, *Streptococcus pneumonia*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Campylobacter jejuni*.

Table 5. MIC and MBC of *C. ladanifer* essential oil against selected bacterial strains.

Bacterial Strains		Concentrations (µL/mL)						MBC/MIC	DMSO
		2	10	20	30	40	50		2 µL/mL
S. aureus	MIC	+	−	−	−	−	−	1	+
	MBC	+	−	−	−	−	−		+
Acinetobacter baumannii	MIC	+	−	−	−	−	−	1	+
	MBC	+	−	−	−	−	−		+
E. coli	MIC	+	−	−	−	−	−	1	+
	MBC	+	−	−	−	−	−		+
Salmonella Typhi	MIC	+	−	−	−	−	−	1	+
	MBC	+	−	−	−	−	−		+

2.6. Antifungal Activity

+: Presence; −: Absence.

The disc diffusion technique enabled us to demonstrate the antifungal activity of *C. ladanifer* essential oil against 10 different fungus strains (**Figure 3**). The antifungal effect of a volume of 5 µL, 10 µL, and 15 µL of *C. ladanifer* essential oil is presented in **Table 6**. The essential oil demonstrated a good inhibitory activity with a slight dose-dependent activity. Fluconazole used as a positive control presented the best inhibition zone diameters against all the selected strains. In relation to the essential oil activity, *C. tropicalis* and *C. neoformans* were the most sensitive with an inhibition zone of 13 mm, followed by *R. rubra* and *Penicillium* sp., which have the same inhibition diameter of 12 mm, then *C. dubliniensis* and *C. glabrata* with inhibition zones of 11 mm. No studies on the antifungal activity of *C. ladanifer* of our study area (Middle Atlas, Morocco) have been carried out, except for a few attempts that have been reported by Boukil et al. [16].

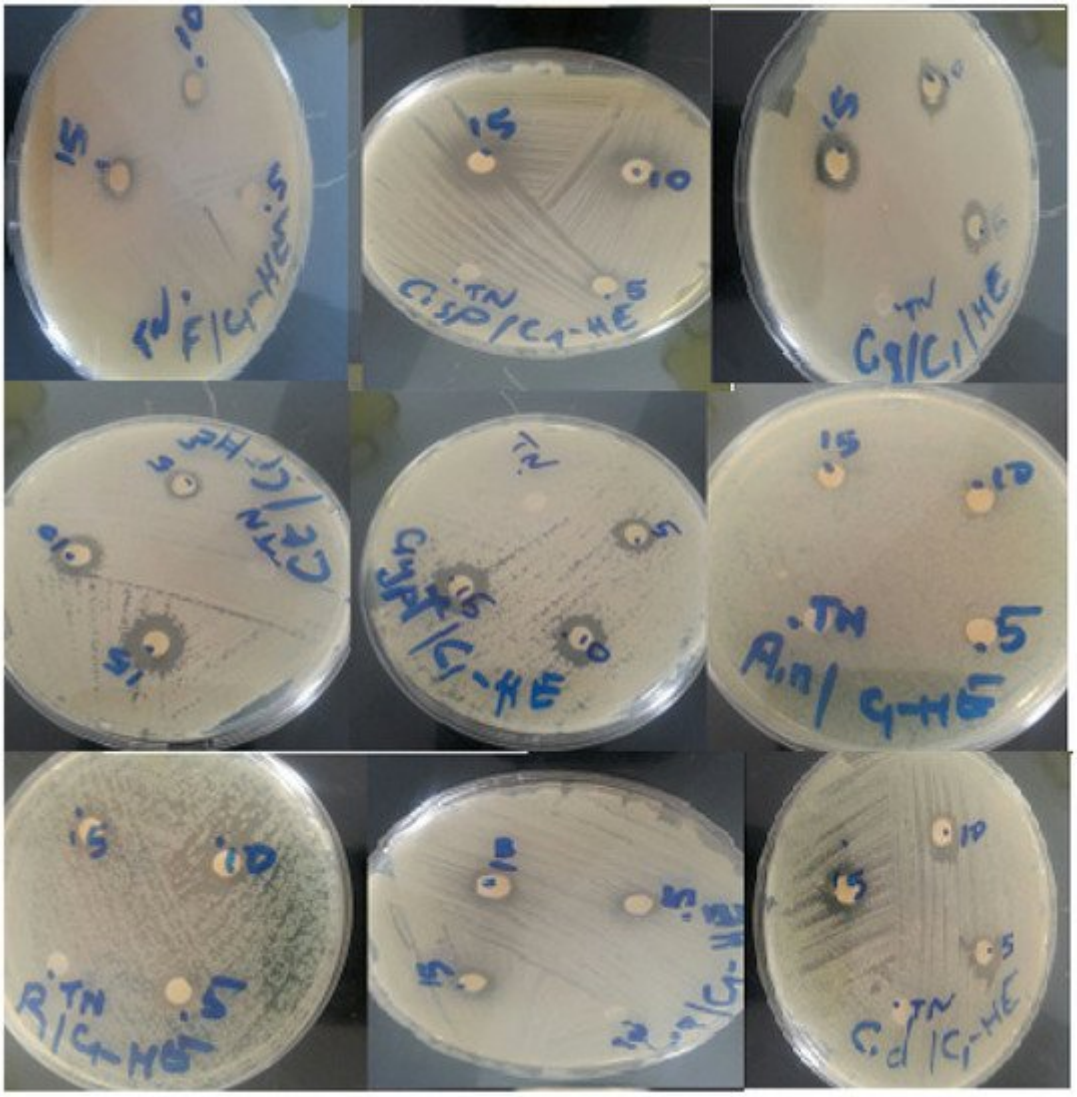

Figure 3. Fungal strains zone of inhibition diameters of essential oil of *C. ladanifer*.

Table 6. Values of the inhibition diameters zone of the essential oil of *C. ladanifer*.

Fungal Strains	Growth Inhibition Diameter (GID) (mm)			Fluconazole GID (mm)
	5 µL	10 µL	15 µL	150 mg
C. albicans	7	8	10	24.7
C. tropicalis	9	11	13	25
C. glabrata	9	11	11	23.7
C. dubliniensis	8	10	11	19.3
Candida sp.	7	8	10	24
R. rubra	8	10	12	18.7
A. niger	-	7	8	13.7

Fungal Strains	Growth Inhibition Diameter (GID) (mm)			Fluconazole GID (mm)
	5 µL	10 µL	15 µL	150 mg
C. neoformans	9	11	13	29.3
Penicillium sp.	7	10	12	13.8
Fusarium sp.	-	7	8	16.3

C)

gainst the ten tested fungal strains. The essential oil of *C. ladanifer* showed an antifungal activity on all fungal strains tested with concentrations ranging between 16 and 64 µL/mL.

Table 7. The effect of the *C. ladanifer* essential oil on the MIC and MFC of the selected fungi strains.

	Fungal Strains		
	MIC	MFC	MFC/MIC
Candida albicans	32	32	1
Candida tropicalis	64	64	1
Candida glabrata	32	32	1
Candida dubliniensis	32	32	1
Candida sp.	16	62	4
Rhodotorula rubra	32	32	1
Cryptoccocus neoformans	64	64	1
Penicillium sp.	64	64	1
Fusarium sp.	64	64	1
Aspergillus niger	32	32	1

The results of the MFC were all compared to those of MIC except for *Candida* sp. strain. Those obtained results were interesting and in agreement with those obtained by Guinoiseau et al., Rossi et al., Vieira et al. which indicate that the essential oil from *C. ladanifer* has an antimicrobial activity on fungi (such as *Aspergillus niger*, *Botrytis cinerea*, *Mucorracemosus,* and *Verticilliumalboatrum*) [38][39][40].

3. Discussions on Essential Oil from Cistus ladanifer L.

This study, among others, focused on revealing the potential bioactivity of the essential oil of *C. ladanifer*. The study's novelty was that plant material was collected during its time of flowering and in a different region (Middle Atlas), which is known for its semi-arid climate. The edaphic and climate parameters and others have influenced the variation of the composition revealed in the chromatographic analysis. The essential oil major components were viridiflorol (17.64%), *trans*-pinocarveol (11.02%), bornyl acetate (9.38%), and ledol (8.85%) indicating the

domination of the oxygenated sesquiterpenes (34.02%), oxygenated monoterpenes (33.14%) in the overall composition.

The oxygenated sesquiterpenes and monoterpenes are a well-known group of compounds with antibacterial and antifungal proprieties [41][42] and their presence among the EO composition explains the majority of the outstanding outcomes obtained.

The strains chosen for this research are of great interest in the areas of clinical and public health. Their increasing resistance to conventional drugs has prompted further research into new, more effective options, particularly natural products [43][44]. *S. aureus* (Gram-positive) is a member of the indigenous human microflora and may be found asymptomatically in a variety of bodily locations. Diseases caused by transmission from these locations are both endemic and epidemic [45]. Infection with *S. aureus* is a leading cause of skin, soft tissue, respiratory, bone, joint, and endovascular diseases. Many strains of *S. aureus* are becoming resistant to current antibacterial treatments, posing a significant issue in medical microbiology [46].

S. Typhi, one of the representatives of the *Salmonella* family, is the direct causative organism of typhoid fever [47] (accompanied by weakness, headaches, mild vomiting, abdominal pain, and constipation). Symptoms may persist for weeks or months if not treated [48].

E. coli usually colonizes human babies' gastrointestinal tracts within a few hours after birth. *E. coli* and its human host often live in excellent health and mutual benefit for decades [48]. These commensal *E. coli* strains seldom cause illness unless the host is immunocompromised or the usual gastrointestinal barriers are broken, as in peritonitis. Diarrhea induced by *E. coli* infection is a growing issue in both the developing and developed worlds, with significant rates of death in newborn infants and animals [49]. Although most commensal representatives found in human and animal gut flora are non-pathogenic, certain strains are very dangerous.

The genus Acinetobacter, over the past 30 years, has experienced considerable taxonomic evolution. Its most prominent example, *A. baumannii*, has emerged as one of the most problematic infections for healthcare facilities worldwide [50]. *A. baumannii* strains resistant to all known antibiotics have now been discovered, indicating a sentinel occurrence that should be addressed by the worldwide health care community as soon as possible. It often attacks the most susceptible hospitalized patients, those who are severely sick and have compromised skin integrity and airway protection [51].

In terms of fungus, non-albicans candida species are increasingly being reported as both colonizers and pathogens causing nosocomial fungal bloodstream infections, accounting for nearly half of all non-superficial candida infections, with *C. glabrata*, *C. tropicalis*, and *C. dubliniensis* being the most common [52].

One other life-threatening fungi exploited in this study is *C. neoformans* which is responsible for cryptococcal meningitis, the most prevalent type of cryptococcosis, often chronic and deadly if left untreated [53]. This virulence is none less than those presented by other fungi such as *Aspergillus niger*, a fungus that causes the "black mold"

on certain fruits and vegetables (contaminant of food) which its consumption (as it secretes ochratoxins – mycotoxins) causes nephrotoxicity and renal tumors [54]. The EO of *C. ladanifer* and through this study demonstrated a strong and real potential that could be better exploited to fight against the threats presented by all the microbial strains studied with slight differences in terms of efficacy.

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