Revue Agrobiologia
www.agrobiologia.net
ISSN (Print): 2170-1652
e-ISSN (Online): 2507-7627

CHEMICAL COMPOSITION AND ANTIMICROBIAL PROPERTIES OF ALGERIAN *CEDRUS ATLANTICA*M. ESSENTIAL OILS

BENOUAKLIL Fatouma[1,2*], HAMAIDI-CHERGUI Fella [1],HAMAIDI Mohand Said [1] and SAIDI Fairouz[1]

1. Laboratory of Biotechnologies, Environment and health, Faculty of Natural Science and Life, University, Blida1, Algeria
2. Department of Biological Sciences, University Djilali Bounaama, Khemis Miliana, Algeria

Reçu le 23/05/2017, Révisé le 15/06/2017, Accepté et mis en ligne le 30/06/2017

Abstract

Description of the subject: In the present work, the antimicrobial activity and the phytochemical composition of *Cedrusatlantica* M. essential oils were investigated.

Objective: This study was undertaken to valorise an aromatic and endemic plant of the Algerian flora,in order to find new bioactive natural products.

Methods: *Cedrusatlantica* M. essential oilsobtained by hydrodistillation from wood and leaves were analyzed by GC–MS. The antimicrobial activity of these oils has been evaluated against nine microorganismsusing the paper disk agar diffusion method.

Results: The results revealed the presence of 16 and 13 compounds with a total yield of 2.1% and 0.12% in the essential oils obtained from wood and leaves respectively. These oils contain the same major components β-Himachalene (31.55%, 30.08%), α-Himachalene (15.00%, 16.38%), Longifolene (11.22%, 14.45%) and δ –Cadinene (4.08%, 4.55%). The tested essential oils showed variable degrees of antimicrobial activity. In general, Gram-positive bacteria were found to be more sensitive to the essential oils than Gram-negative bacteria.

Conclusion: The leaves essential oil from *Cedrusatlantica* have a potential to be used as a natural antimicrobial agent against *Enterococcus faecalis*.

Keywords: Antimicrobial activity;*Cedrusatlantica;* essential oils; GC–MS; *Enterococcus faecalis*

COMPOSITION CHIMIQUE ET PROPRIÉTÉS ANTIMICROBIÈNNES DES HUILES ESSENTIELLES DE *CEDRUS ATLANTICA* M. DE L'ALGÈRIE

Résumé

Description du sujet: Dans le présent travail, l'activité antimicrobienne et la composition phytochimique des huiles essentielles de *Cedrus atlantica* M. ont été étudiées.

Objectifs: Cette étude a été entreprise pour valoriser une plante aromatique et endémique de la flore algérienne, afin de trouver de nouveaux produits naturels bioactifs.

Méthodes: Les huiles essentielles de *Cedrus atlantica* M. obtenues par hydrodistillation à partir du bois et des feuilles ont été analysées par GC-MS. L'activité antimicrobienne de ces huiles a été évaluée contre neuf microorganismes par la méthode de diffusion sur gélose en utilisant des disques en papier.

Résultats: Les résultats ont révélé la présence de 16 et 13 composés avec un rendement total de 2,1% et 0,12% dans les huiles essentielles obtenues à partir du bois et des feuilles respectivement.Ces huiles contiennent les mêmes composants principaux β-Himachalène (31,55%, 30,08%), α-Himachalène (15,00%, 16,38%), Longifolène (11,22%, 14,45%) et δ -Cadinène (4,08%, 4,55%).Les huiles essentielles testées présentaient des degrés variables d'activité antimicrobienne. En général, les bactéries Gram-positive se sont montrées plus sensibles aux huiles essentielles que les bactéries Gram-négative.

Conclusion: Les résultats indiquent que l'huile essentielle des feuilles de *Cedrusatlantica* peut être utilisée comme agent antimicrobien naturel contre *Enterococcusfaecalis*.

Mots clés: Activité antimicrobienne; huiles essentielles; *Cedrusatlantica*; GC-MS;*Enterococcusfaecalis*

*Corresponding author: BENOUAKLIL Fatouma Laboratory of Biotechnologies, Environment and health, Faculty of Natural Science and Life, University Blida1, Algeria Email: benouaklil.fetouma@yahoo.fr

INTRODUCTION

Each year, one to three antibiotics are placed on the market [1] because each antibiotic has a limited effective lifetime after which microorganisms develop resistances [2, 3]. The use of plant extracts as antimicrobial agents represents a low risk for the development of resistance by microorganisms because these products are composed of several phytocompounds of different groups [4] which work in synergy [5].Consequently,aromatic and medicinal plants have acquired particular attention in the field of intensive research on the natural antimicrobial compounds. They constitute a constant source of active reagents against pathogen germs [6].

The Atlas cedar (*Cedrus atlantica* M.) belongs to the Pinaceae family [7] is an endemic species of the North African mountains from Morocco and Algeria [6]. According to literature, among important pharmacological properties that support its clinical use, we can name antiseptic, healing, antifungal, purifying and relaxing effects [8].

In terms of previous reports, the chemical composition of different parts of this plant has been widely studied [9, 10, 11, 12, 13, 14, 6]. On the other hand, few studies have been performed on the antimicrobial effect of the essential oils of Morocco's Atlas cedar [13, 6].However, in our knowledge there is no published report on antimicrobial activity of wood and leaves essential oils extracted from Algerian *Cedrus atlantica*M.

The aim of the phytochemical study of *Cedrus atlantica* was to find natural compounds with interesting biological activities based on scientist researches. Therefore, we investigated the possible antimicrobial activity of the essentials oils from wood and leaves of this species growing in abiotic stress in Algerian mountains.

MATERIALS AND METHODS

1. Plant material

The *Cedrusatlantica* M. wood and leaves were collected the morning in the Chréa mountains (Blida, Algeria) at an altitude of 1500 m during the period from September to December 2012.

2. Isolation of the essential oils

Fresh parts of *Cedrus atlantica* M. wood and leaves were cut into small pieces. The essential oil of each sample was obtained by hydrodistillation with a Clevenger Apparatus. The obtained oils were collected for 4 h and preserved under refrigeration (+4°C) in an amber vial until further analysis. The yield of each component was calculated per 100g of plant material. The whole experiment was performed in triplicate.

3. Gas chromatography-mass spectrometry analysis (GC-MS)

GC-MS analysis of the oils were carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m x 0.25 mm, 0.25 μm film thickness) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode. Operating under the conditions as described below: initial temperature 38 °C, maximum temperature 250°C, equilibration time 5 min, ramp 6 °C/min, final temperature 250°C, inlet: split less, pressure 6.75 psi, purge flow 1 ml/min and 1µlof sample was injectedfor analysis, gas type: helium, column: capillary. The components of the oils were identified by matching their mass spectra with those of the computer library (NIST mass spectra library). The percentage composition was calculated from the summation of the peak areas of the total oil composition.

4. Microbial strains

The antimicrobial activity of the essential oils samples was tested towards nine different microorganisms. Three gram negative bacteria, namely *Klebsiellapneumoniae* ATCC 4352, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and five gram positive bacteria namely *Micrococcus luteus*ATCC 533, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 2035, *Bacillus subtilis* ATCC 9372. *Candida albicans* ATCC 10231was the fungus used in this study. The cultures were obtained from the Microbiology Laboratory of the Research and Development

Centre-SAIDAL (Algiers, Algeria), the Antibiotical Complex-SAIDAL (Medea, Algeria) and the Pasteur Institute (Algiers, Algeria). These strains were maintained on slants of Nutrient Agar for bacteria and Sabouraud Agar for the fungus at 4°C.

5. Antimicrobial activity assay

Antimicrobial activities of the essential oils from the different parts of *C. atlantica* were assessed using the paper disk agar diffusion method according to Rios et *al*. [15], Freney *et al*. [16]. and Najjaa *et al*. [17]. Petri plates were prepared with 20 ml of sterile Nutrient Agar and Mueller–Hinton Agar for bacteria and Sabouraud Agar for fungi. The agar plates were stored at 4 °C before being used. Fresh bacterial and fungal inoculums were prepared from over-night cultures to obtain microbial inoculums in the logarithmic growth phase [18]. The overnight culture of each microorganism was diluted to $2x10^6$ CFU/ml in sterile saline solution. The concentration of each suspension used for inoculation was standardized by adjusting the optical density to 0.5 at 570 nm wavelength (spectrophotometer UV/visible, Shimadzu UVmini 1240). Absorbent disks (6 mm diameter) were impregnated with 10 µl of different essential oils and then placed on the surface of inoculated plates (90 mm) and incubated at 37°C during 24 h for bacteria and 25°C during 48h for the fungus. Antimicrobial activity was evaluated by measuring the inhibition zone which is the diameter of the zone visibly showing the absence of growth, including the 6 mm of disk. All the tests were performed in triplicate.

RESULTS

1. Yield and chemical composition of essential oils

The yields of essential oils of the different *Cedrus atlantica* M. parts are ranged from 0.12% ±0.01 to 2.1%±0.05 (w/w), on a dry weight basis. The highest yield was obtained from the wood while the leaves gave the lowest yield.

Upon GC-MS analysis, the wood essential oil was found to contain 16 different compounds, representing 76.31% of the total oil. The identified compounds are listed in table 1. According to their elution order on a HP-5MS 5% phenylmethylsiloxane capillary column. The major compounds detected were β-Himachalene (31.55%), α-Himachalene (15.00%), Longifolene (11.22%) and δ – Cadinene (4.08%). Thirteen compounds consisting up to 70.46% of the leaves essential oil were identified by GC/MS analysis. This essential oil was consisted mostly ofβ-Himachalene (30.08%), α-Himachalene (16.38%), Longifolene (14.45%) and δ – Cadinene (4.55%). The major compounds of this oil are the same as those of the wood essential oil with the same order of predominance and slight percentages variations. Essential oils from wood and leaves of the Atlas cedar are dominated by terpenic hydrocarbons with the following percentages 72.45% and 69.09% (Table 1).

2. Antimicrobial activity

The in vitro antimicrobial activity of the different essential oils of *C. atlantica* against the employed bacteria and fungus was qualitatively assessed by the presence or absence of inhibition zones. According to results given in Table 2.a total of nine microorganisms, including five Gram-positive bacteria, three Gram-negative bacteria and one fungus were tested.

The antimicrobial activity of essential oils varies depending on the tested strains. The five following microbial strains: *Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis* and *Bacillus subtilis* are sensitive to the tow essential oils tested with a greater sensitivity to the leaves essential oil.

Enterococcus faecalis ATCC 2035 is the most sensitive Gram-positive microorganism to the leaves essential oil (22.96 mm ± 0.60).This inhibition zone is close than that of Chloramphenicol 30 mcg (22.78 mm ± 0.36).

Table 1:*C. atlantica* woods and leaves essential oils compounds identified by GC–MS

N°	Compounds[a]	Composition (%)	
		Wood	Leaves
1	α- Pinene	0.06	0.05
2	D-Limonene	_	0.02
3	Limonaketone	1.33	1.30
4	Borneol	_	0.02
5	α-Terpineol	_	0.04
6	α-Longipinene	0.16	_
7	Isolongifolene, 4,5,9,10-dehydro-	1.34	_
8	δ-selinene	1.88	_
9	α-Cedrene	0.34	0.25
10	Cedrene	_	0.08
11	Himachala-2,4-diene	1.22	0.81
12	α-Himachalene	15.00	16.38
13	Longifolene	11.22	14.45
14	β-Himachalene	31.55	30.08
15	Isolongifolene	2.27	2.44
16	δ -Cadinene	4.08	4.55
17	Cadina-1(10),6,8-triene	1.09	_
18	Cadala-1(10),3,8-triene	2.25	_
19	3-Isobutyl-4,5-dimethyl-3H-isobenzofuran-1-one	1.15	_
20	Cubenol	1.38	_
21	Phyllocladene	0.06	_
Monoterpenes%		1.39	1.43
Sesquiterpenes%		74.92	69.03
Diterpenes%		0.00	0.00
TerpenicHydrocarbon		72.45	69.09
TerpenicAlcohol		1.38	0.07
TerpenicKetone		1.33	1.30
Terpenic Ester		1.15	0.00
Total detected (%)		76.31	70.46

Compounds listed in order of elution from HP-5MS column

Table 2: Zones of growth inhibition (mm) showing antimicrobial activity of *C. atlantica*essential oils and standard antibiotic

	Inhibition zone diameter[a] (mm)			
	C. atlantica essential oils		CH	AMB
Microorganisms	Wood	Leaves		
Gram +				
M. luteus	13.60±0.23	13.29±0.05	32.04±0.68	NA
S. aureus	9.78±0.14	10.85±0.72	22.34±0.2	NA
S. epidermidis	10.07±0.02	12.54±0.68	28.55±0.30	NA
E. faecalis	12.02±0.12	22.96±0.60	22.78±0.36	NA
B. subtilis	9.56±0.28	14.08±0.66	31.82±0.42	NA
Gram-				
K. pneumoniae	ND	9.75±0.31	34.12±0.47	NA
E. coli	ND	8.27±0.24	26.23±0.07	NA
P. aeruginosa	7.18±0.04	ND	13.99±0.75	NA
Yeast				
C. albicans	ND	9.15 ±0.33	NA	9.15±0.15

NA: Not applicable

ND: Not detected

CH: Chloramphenicol 30µg/disc; AMB: Amphotericin B 10µg/disc

[a]Results are presented as mean ± standard deviation (N = 3)

DISCUSSION

It is interesting to note that the yield of wood is relatively higher than those of some plants industrially exploited as an essential source of oil. Such as thyme (1%) [19] and juniper (1.62%) [14]. The hydrodistillation of *C. atlantica* M. wood provides an essential oil yield lower than that obtained by Satrani *et al.* [13] in their work on the wood of the same specie of the Central Middle Atlas of Morocco (2.78%). This can be explained by the period of the woodcutting (after flowering). The works of Derwich *et al.* [14] have shown that the leaves oil rate of Atlas cedar from Morocco (Boulmane region) which is 1.82% is significantly higher than that of our study. This can be explained by the different period of harvest (before the appearance of catkins). As reported by McGimpsey and Douglas [20]; Salgueiro *et al.* [21] and Gilles *et al.* [18], climate, genotype, growth location, rainfall and harvesting regime all can affect the total essential oil content of plants.

The studies done by Dahoun *et al.* [9]; Aberchane *et al.* [10] ; Boudarene *et al.* [12] and Satrani *et al.* [13] on essential oils of Atlas cedar wood of different regions reveal that there is a qualitative difference for some major compounds with dominance of some compounds generally in common as the β-Himachalene and the α-Himachalène. The results obtained by Derwich *et al.* [14] analysis on the leaves essential oil of the Atlas Cedar from Morocco (Tichoukt Region) revealed some qualitative differences with our results with noting the existence of two major compounds in common. These have dissimilar proportions and are respectively 9.89% against 30.08% for the β-Himachalene and 4.15% against 16.38% for the α-Himachalène.The leaves essential oil of the Atlas cedar was also the subject of two other works. That of Lahlou [11]conducted on a sample from Rabat (Morocco) and that of Boudarene *et al.* [12]conducted on a sample from the Bainem forest (Algeria). In both studies the major compounds of the essential oil are totally different from ours. The variations found in the chemical composition of essential oils, qualitatively and quantitatively depend on certain environmental factors, the plant part used, the age, the period of its growth cycle, or even genetic factors [22, 23, 24, 25, 26]. Also, the observed difference between the chemical composition of the essential oils of the Moroccan Atlas cedar and those of Algeria could be due to the difference of both climatic and geographical factors, such as the altitude and the soil type [27, 28, 29, 7].

Following the outcome of our biologic tests, we see that the Atlas cedar essential oils has manifested various antibacterial and antifungal characteristics depending on the essential oil composition of the vegetable organ in question (wood or leaves) and tested microorganisms. According to Satrani *et al.* [13], the essential oils components are classified depending on their antimicrobial potency in the following descending order: Alcohol> ketones> Hydrocarbons . There is some evidence that minor components have a critical part to play in antibacterial activity, possibly by producing a synergistic effect between other components [30].

The study done by Satrani *et al.* [13] on the antibacterial effect of the essential oil of Morocco's Atlas cedar wood showed that it inhibits the growth of *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*. This is consistent with our results except for the last seed.

It is commonly known that Gram negative bacteria are more resistant to the essential oils [31]. Our essential oils have not escaped this finding, Gram positive bacteria have been much more sensitive compared to gram negative bacteria. An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable [32, 33]. Leakage of ions and other cell contents can then occur [34, 35, 36, 37, 38, 39, 40, 41]. Al-though a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death [42].

CONCLUSION

The leaves essential oil could be an additional means to fight against nosocomial infections caused by *E. faecalis* which is resistant to many commonly used antimicrobial agents.

Further studies are needed for more extensive assessment of other biological activities of each essential oil separately or in combination.

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