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Full Length Article

Chemical Composition and *In Vitro* Antibacterial Activity of the Essential Oil of *Cedrus atlantica*

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ABSTRACT

This work was conducted to determine the phytochemistry and antibacterial activity of the essential oil from leaves of *Cedrus atlantica* growing in Morocco. The chemical constituents of the essential oil obtained from the leaves of *Cedrus atlantica* in the Atlas median, mountain region of Morocco was determined by hydro-distillation, analysed by gas chromatography equipped with flame ionisation detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS). The antimicrobial activities were tested *in vitro* on gram-negative bacteria *Escherichia coli, Pseudomonas aeroginosa* and *Klebsiella pneumonia* and gram-positive *Staphylococcus aureus, Enterococcus faecalis, Bacillus sphericus* and *Staphylococcus intermedius* using agar disc diffusion and minimum inhibitory concentration (MIC). Thirty one compounds were identified in leaves oil representing 92.40% of the total oil composition. The yield of essential oil of *Cedrus atlantica* was 1.82% and the major compound in aerial parts was α-pinene (14,85%) followed by himachalene (10.14%), β-himachalene (9,89%), σ-himachalene (7,62%), cis-α-atlantone (6,78%), himachalol (5,26%) and α-himachalene (4,15%), germacrene D (3.52%), β-caryophyllene (3.14%), cadinene (3.02%), β-pinene (2.35%), humulene (2.30%) and copaene (2.26%). The bacterial strains tested were found to be sensitive to essential oil studied and showed very effective bactericidal activity with the strongest inhibition zone: 12 at 25 mm and 6 at 22 mm for Gram-negative and Gram-positive bacteria, respectively.

Key Words: *Cedrus atlantica;* Essential oil; GC/MS; α-pinene; Antibacterial activity

INTRODUCTION

Medicinal plants have been traditionally used for pharmaceutical and dietary therapy in long history. A number of herbs and many relevant prescriptions have been screened and used for treating and preventing various tumours and inflammations as folk practices. Essential oils used in traditional therapies are also called volatile oils are generally aromatic oils obtained by the steam or hydro distillation of plants. Different parts of plants have been used to obtain essential oils. Essential oils and their components are widely used in medicine as constituents of different medical products in the food industry as flavouring additives and also in cosmetics as fragrances (Cowan, 1999) and pharmaceutical industries (Reische *et al*., 1998). These include flowers, leaves, seeds, roots, stems, bark and wood though secretionary parts. Essential oils are generally used in the cosmetic, medical and food industries. Antibiotic resistance has become a global concern (Westh *et al*., 2004). Moreover screening of such plant extracts for antimicrobial activity has always been of great interest to scientists looking for new sources for drugs for the treatment of various diseases (Sökmen *et al*., 1999; Oka *et al*., 2000). In this study, *Cedrus atlantica* was investigated for their efficacies against MMP-13 inhibitory activity. This species, only distributed in Morocco and Algeria, possesses a high quality wood, used in construction and handicraft industries. The essential oil of *Cedrus atlantica* has been shown to possess antifungal (Bouchra *et al*., 2003), antimicrobial (Hammer *et al*., 1999), antiviral (Monica *et al*., 2008), molluscicidal (Lahlou, 2003) and anti-inflammatory (Sugita *et al*., 2004) activities. Identification and quantification of potent bioactive compounds from these plants were also performed.

MATERIALS AND METHODS

Plant material: The aerials parts of *Cedrus atlantica* were

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collected in August 2009 at Tichoukt (15 km in the south east of Boulmane) a mountainous region from Morocco. Voucher specimens are available for inspection at the herbarium of the Scientific Institute of the University of Mohamed V, Rabat (Morocco). The coordinates: latitude: 35° 42 '21" longitude: 4° 32' 31"; altitude: 1000 m. The climate is semi-humid with strong continental influence with an annual average temperature of 20°C. The leaves were then isolated from the other specimen and conserved for extraction.

Essential oil extraction: The leaves of examined plants were dried in shadow at room temperature and immediately hydro-distilled (30 g) for 2.5 h using a modified Clevenger-type apparatus. The yellowish oil (1 mL) for leaves was dissolved in hexane and then dried over anhydrous sodium sulfate. After filtration, the solvent was removed by distillation under reduced pressure in a rotary evaporator at 35°C and the pure oil kept at 4°C in the dark, until the moment of analysis.

Chromatographic (GC/MS & GC-FID) analysis: The chemical composition of leaf oil from *Cedrus atlantica* in Morocco was determined by GC- FID (TRACE GC-ULTRA S/N 20062969, Thermo Fischer) and GC-MS (TRACE GC-ULTRA S/N 20062969-PolarisQ S/N 210729, Thermo Fischer) equipped with TRIPLUS AS S/N 20063460 in the light of the following experimental protocol.

Gas chromatography analysis (GC-FID): The quantitative analysis was done with the help of a chromatographer in gas phase equipped with flame ionisation detector (GC-FID), Varian capillary column (5% poly diphenyl 95% dimethylsiloxane, TR5- CPSIL- 5CB; 50 m length, 0.32 mm of diameter & Film thickness 1.25 µm). The column temperature was programmed from 40 to 270°C for 4°C/min and finally held at that temperature for 10 min. The temperature of the injector was fixed to 250°C and the one of the detector (FID) to 280°C. The debit of gas vector (nitrogen) was fixed to 1 mL/min and split injection with split ratio 1:40. The volume of injected was 1 µL of diluted oil in hexane solution (10%). The percentage of each constituent in the oil was determined by area peaks.

Gas chromatography-mass spectrometry analysis (GC/MS): The identification of different chemical constituents was done by gas phase chromatography (TRACE GC-ULTRA) coupled with spectrometer (PolarisQ). The utilised column was; Varian capillary column (TR5- CPSIL- 5CB; 50 m length, 0.32 mm of diameter & Film thickness 1.25 µm). The column temperature was programmed from 40 to 280°C for 5°C/min. The temperature of the injector was fixed to 260°C and the one of the detector (PolarisQ) to 200°C. The ionization mode was Electron Impact E I. (70 eV). The scan range was between 40 and 650 amu. The debit of gas vector (Helium) was fixed to 1.5 mL/min. The volume of injected specimen was 1 µL of diluted oil in hexane. The software utilized was x-calibur (Thermo Fisher) with NIST-MS library. The constituents of essential oils were identified in comparison with their Kovats Index, calculated in relation to the retention time of a series of lineary alkanes (C_4-C_{28}) with those of reference products and in comparison with their kovats Index with those of the chemical constituents gathered by Adams (2001) and in comparison with their spectres of mass with those gathered in a library of (NIST-MS) type and with those reported in the literature (Woerdenbag *et al*., 1993; Palá-Paúl *et al*., 1999).

Antibacterial activity: The minimal inhibition concentration (MIC) values of essential oils were evaluated according to published procedures (Koneman *et al*., 1997; Iscan *et al*., 2002; Guven *et al*., 2005; Derwich *et al*., 2010). The minimal inhibitory concentration (MIC) was determined only with micro-organisms that displayed inhibitory zones. MIC was determined by dilution of the essential oils in dimethyl sulfoxide (DMSO) and pipetting 0.01 mL of each dilution into a filter paper disc. Dilutions of the oils within a concentration range of 0.25-1.62 mg/mL were also carried out. MIC was defined as the lowest concentration that inhibited the visible bacterial growth (NCCLS, 2005). A negative control was also included in the test using a filter paper disc saturated with DMSO to check possible activity of this solvent against the bacteria assayed. The experiments were repeated at least twice.

In this work, antibacterial activity of *Cedrus atlantica* oil was examined using different bacterial species. In addition, chemical composition of volatile constituents, were also determined.

RESULTS AND DISCUSSION

Chemical composition of the essential oil: The compounds essential oil of *Cedrus atlantica* from Morocco is listed in order of their elution on the (TR5- CPSIL- 5CB column) (Fig. 1). The GC/MS analysis of essential oil leaves extracteds from *Cedrus atlantica* revealed the presence of Thirty one compounds, representing 92.40% of the total composition (Table I). The major group of compounds, the main one being α-pinene (14,85%) followed by himachalene (10.14%), β-himachalene (9,89%), σ-himachalene (7,62%), cis-α-atlantone (6,78%), himachalol (5,26%) and α-himachalene (4,15%), germacrene D (3.52%), β-caryophyllene (3.14%), cadinene (3.02%), β-pinene (2.35%), humulene (2.30%) and copaene (2.26%).

The essential oil yield of *Cedrus atlantica* collected from region of Boulmane (Morocco) was 1.82%; it's relatively higher than other plants industrially exploited as a source of essential oils: Thymus (1%) (Imelouane *et al*., 2009), lavender (0.8-2.8%), menthe (0.5-1%), néroli (0.5-1%) and Laurel (0.1-0.35%) (Edward *et al*., 1987), Tetraclinis (0.22%) (Bourkhiss *et al*., 2000), *Juniperus phoenicea* (1.62%) (Derwich *et al*., 2010) and *Lippia rotundifolia* (0.01%) (Suzana *et al*., 2008). Contrary to the oils yield of leaves of *Cedrus atlantica* study in algeria,

Table I: Chemical composition of leaves of essential oil of *Cedrus atlantica* from Morocco

Peak	Constituents	*RT (min)	**Air (%)
1	Trans-pinocarveol	4.85	0.11
2	α-Terpinene	5.15	1.02
3	Camphene	7.79	0.23
4	α-Cubebene	10.80	0.50
5	Cis-ocimene	13.42	1.62
6	Humulene	19.96	1.30
7	β-Caryophyllene	19.98	3.14
8	σ-Himachalene	20.41	7.62
9	cis-α-Atlantone	20.50	6.78
10	Himachalol	22.00	5.26
11	Myrcene	22.50	0.15
12	α-Himachalene	23.01	4.15
13	α-Pinene	23.65	14.85
14	β-Pinene	24.50	1.35
15	Himachalene	24.54	10.14
16	Cadinene	25.18	3.02
17	Isocaryophillene	26.50	1.10
18	β-Himachalene	27.33	9.89
19	Germacrene-D	28.15	3.52
20	β-Copaene	28.36	2.26
21	Cymene	28.50	1.05
22	3-Carene	28.64	1.10
23	Verbenol	31.51	2.24
24	Limonene	31.74	2.01
25	Ylangene	34.85	2.20
26	β-Phellandrene	38.40	2.19
27	γ-Amorphane	40.22	2.22
28	Terpinen-4-ol	42.35	0.55
29	Terpinolene	45.26	0.38
30	α-Terpinenol	45.85	0.35
31	Myrtenal	50.89	0.10
Total Identified Constituents			**92.40**
Yields (%)			**1.82**

*RT: Retention time obtained by chromatogram (Fig. 1)
Air (%):** was determined by mass spectrometry (PlarisQ)

Table II: Antibacterial activity of leaves essential oils of *Cedrus atlantica* from Morocco

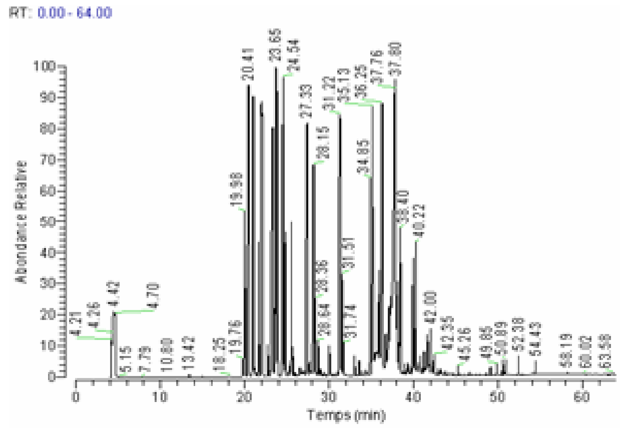
Bacteria	Micro-organisms	Disc diffusion assay (inhibition zone mm)	MIC (mg/mL)
Gram-negative	*Escherichia coli*	25	0.25
	Pseudomonas aeroginosa	21	0.98
	Klebsiella pneumoniae	12	1.45
Gram-positive	*Staphylococcus aureus*	22	0.68
	Staphylococcus intermedius	19	1.25
	Enterococcus faecalis	11	1.31
	Bacillus sphericus	6	1.62

Disc diameter 6 mm average of two consecutive trials
MIC: Minimal Inhibitory Concentration, concentration range 0.25-1.62 mg/Ml

which is (1.2%) in Ouled Yacoub in the Aures region (East of Algeria) and TaIa Guilef (1.7%) in the Djurdjura region (central Algeria) (Boudarene *et al*., 2004).

The chemical compositions revealed that this leaves had compositions relatively similar to those of other *Cedrus atlantica* essential oils analyzed by Teisseire and plattier (1974), which the major compounds was himachalene and it's relatively similar to the composition of essential oil of leaves of *Cedrus atlantica* study in Lebanon, which the major constituents were himachalol (22.50%), β-himachalene (21.90%) and α-himachalene (10.50%) (Monica *et al*., 2008). Contrary to the composition of essential oils of leaves of *Cedrus atlantica* study in Egypt, which main constituents were: α-pinene (37.1-5.5%), β-pinene (8.6-1.9%), myrcene (3.6-0.6%), limonene (2.5-0.6%), bornyl acetate (5.4-4.0%), (E)-β-farnesene (6.8-1.9%) and manool (8.3-20.7%) (Boudarene *et al*., 2004). Intensive research on the chemical characteristics has been conducted on this species (Bean, 1992; Cassady & Whitley, 1997; Whitley *et al*., 1998). In this study, the total oil composition of essential oils of *Cedrus atlantica* collected from Tichoukt mountainous region (Morocco), where 92.40%, it's relatively higher than other plants study in Turkey, which the major constituents were α-pinene (24.78%), abieta-7,13-diene (16.67%), abieta-8,11,13-triene (6.85%), manool (5.83%), terpinen-4-ol (3.74%), α-terpineol (3.42%), p-cymene (2.89%) and limonene (2.69%) (Necmettin *et al*., 2005).

Fig. 1: Chromatogram of *Cedrus atlantica* from Morocco



Antibacterial activity: In recent years, there has been target interest in biologically active constituents, isolated from plant species for the elimination of pathogenic micro-organisms, because of the resistance that micro-organisms have built against antibiotics (Essawi & Srour, 2000) or because they are ecologically safe compounds (Lee *et al*., 2005). Essential oils have been widely used in traditional medicine. Among others, antibacterial, antifungal, immunomodulatory, antinflammatory and antirheumatic activities have been described (Saller *et al*., 1995; Reichling, 2001).

The results obtained in the antibacterial activity study of the leaves essential oils of *Cedrus atlantica* from Morocco are shown on Table II. With the agar disc diffusion assay, oils were found to be active *Escherichia coli*, *Pseudomonas aeroginosa, Staphylococcus aureus* and *Staphylococcus intermedius* at a minimal inhibitory concentration (MIC) of 0.25, 0.98 0.68 mg/mL and 1.25 mg/mL. Against *Klebsiella pneumoniae, Enterococcus faecalis* and *Bacillus sphericus* the oil from the leaves was found to be more active; the oils showed MIC values of

1.45, 1.31 and 1.62 mg/mL, respectively. The data indicated that *Escherichia coli*, *Pseudomonas aeroginosa* and *Staphylococcus aureus* were the most sensitive strain tested to the oil of *Cedrus atlantica* with the strongest inhibition zone 25, 21 and 22 mm, respectively. The *Klebsiella pneumoniae, Staphylococcus intermedius* and *Enterococcus faecalis*, were found to be more sensitive among bacteria with inhibition zone of 12, 19 and 11 mm, respectively. Modest activities were observed against *Bacillus sphericus* with inhibition zones of 6 mm.

Essential oils rich in α-pinene demonstrated potential antibacterial activity (Hajji *et al*., 1993; Tantaoui-Elaraki *et al*., 1993). The major components of this oil, α-pinene, have been known to exhibit antimicrobial activity against the bacterial strains (*Escherichia coli, Staphylococcus aureus, Micrococcus luteus* & *Bacillus subtilis*) (Bourkhiss *et al*., 2000). Monoterpenes hydrocarbons, terpinenes, have also shown antimicrobial properties that appear to have strong to moderate antibacterial activity against Gram positive bacteria (Oyedeji & Afolayan, 2005). The bridged bicyclic monoterpenes *α*-pinene and *β*-pinene showed considerable biological activity (Delaquis *et al*., 2001; Kim *et al*., 2003). The antimicrobial activities revealed that this leaves had similar to those of *Juniperus* essential oils analyzed by Angioni *et al*. (2003), which the major component was α-pinene (86%) and *Cedrus libani* was recently described that cones and leaves of *Cedrus libani* possess antimicrobial activity (Digrak *et al*., 1999). Furthermore the cones of *Cedrus libani* possess antiulcerogenic remedies for anti-Helicobacter pylori activity (Yesilada *et al*., 1999). The antimicrobial activity of the ethanol extract of resins obtained from the roots and stems of *Cedrus libani* was also investigated. Results revealed that *Cedrus libani* resins are highly effective against tested micro-organism by preventing their growth to a greater extent (Kizil *et al*., 2002). The antimicrobial activities have been mainly explained through C_{10} and C_{15} terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bonds with active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils (Belletti *et al*., 2004). On the other hand, enantiomers of α-pinene, β-pinene, limonene and linalool have a strong antibacterial activity (Magiatis *et al*., 1999). Pinene-type monoterpene hydrocarbons (α-pinene & β-pinene) are well-known chemicals having antimicrobial potentials (Dorman & Deans, 2000).

CONCLUSION

The leaves oil obtained from *Cedrus atlantica* revealed a wide variety (n=31) of volatile constituents, which made up 92.40% of the total essential oil. The essential oil yields of the studies were 1.82%. The major compounds were α-pinene (14,85%) followed by himachalene (10.14%), β-himachalene (9,89%), σ-himachalene (7,62%), cis-α-atlantone (6,78%), himachalol (5,26%) and α-himachalene (4,15%), germacrene D (3.52%), β-caryophyllene (3.14%), cadinene (3.02%), β-pinene (2.35%), humulene (2.30%) and copaene (2.26%). *Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis, Bacillus sphericus* and *Staphylococcus intermedius* were found to be sensitive to essential oils of *Cedrus atlantica.*

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