

Full Length Research Paper

# Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L.

Khadidja Mazari<sup>1</sup>, Nassima Bendimerad<sup>1</sup>, Chahrazed Bekhechi<sup>1</sup> and Xavier Fernandez<sup>2\*</sup>

<sup>1</sup>Université Abou Bekr Belkaid Tlemcen, Laboratoire des Produits Naturels, Département de Biologie Moléculaire et Cellulaire, Nouveau Pôle Rocade II Tlemcen 13000, Algérie.

<sup>2</sup>Université de Nice Sophia-Antipolis, Laboratoire de Chimie des Molécules Bioactives et des Arômes, UMR CNRS 6001, Institut de Chimie de Nice, Parc Valrose 06108 Nice cedex 2, France.

Accepted 26 April, 2010

The chemical composition of essential oils isolated from the leaves by steam-distillation of Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L. were analyzed by GC-MS. The oils were predominantly composed of monoterpene hydrocarbons (72.9 and 75.7%), with  $\alpha$ -pinene as major constituent (34.5 and 60.5%).  $\beta$ -phellandrene (22.4%) and  $\alpha$ -Terpinyl acetate (14.7%) were the second most important constituents of the *J. phoenicea* oil. While cedrol (8.3%) was found to be the second most important constituent in the oil of *C. sempervirens*. The antimicrobial activity of the essential oils was evaluated against five bacteria (3 Gram-positive and 2 Gram-negative), and 3 fungi. Results showed that the oils exhibited moderate antibacterial and antifungal activities.

**Key words:** *Juniperus phoenicea* L., *Cupressus sempervirens* L., Cupressaceae, essential oils, GC-MS, antimicrobial activity.

## INTRODUCTION

In the last years, scientists have focused on increasing human infections caused by pathogen bacteria and fungi. Microorganisms have unfavourable effects on the quality and safety of life. Synthetic chemicals are widely used against these microorganisms; unfortunately they develop resistance to many antibiotics due to the indiscriminate use of commercial antibiotics (Service, 1995; Mukherjee et al., 2002). In addition, these antibiotics sometimes cause allergic reaction and immunity suppression. Currently, people heal a lot and although in many cases they are turning to synthetic drugs, but a vast majority is turning to natural products. Therefore the use of essential oils is less damaging to the human health (Isman, 2000; Misra and Pavlovstathis, 1997) because they are generally few toxic and they do not have side effects. On the other hand, the food industry at present

is facing a tremendous pressure from consumers for using chemical preservatives to prevent the growth of food borne and spoiling microbes. To reduce or eliminate chemically synthesized additives from foods is a current demand worldwide. A new approach to prevent the proliferation of microorganisms is the use of essential oils as preservatives. Essential oils of plants are of growing interest both in the industry and scientific research because of their antibacterial and antifungal properties and make them useful in many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Reynolds, 1996; Lis-Balchin and Deans, 1997).

The species *Juniperus phoenicea* is considered as an important medicinal plant largely used in traditional medicine. Its leaves are used in the form of decoction to treat diarrhea, rheumatism (Bellakhder, 1997) and diabetes (Bellakhder, 1997; Allali et al., 2008). The mixture of leaves and berries of this plant is used as an oral hypoglycaemic agent (Amer et al., 1994), whereas the leaves are used against bronco-pulmonary disease

\*Corresponding author. E-mail: [xavier.fernandez@unice.fr](mailto:xavier.fernandez@unice.fr). Fax: 33-(0) 492076125.

and as a diuretic (Bellakhder, 1997). *Cupressus sempervirens* L. has been traditionally used for the treatment of colds, flu, evils throat and rheumatism (Larousse, 2001). The brunches of this plant are used as antiseptics and antispasmodics (Bellakhder, 1997).

The geographic area of *Cupressus* genus is limited to the northern hemisphere and many species have been studied (Zavarin et al., 1971; Pierre-Leandri et al., 2003). To the best of our knowledge, there are many papers report on the chemical composition of essential oils of *J. phoenicea* and *C. sempervirens* grown in north Mediterranean basin (Chanegriha et al., 1993, 1997; Rezzi et al., 2001; Tapondjou et al., 2005). In the southern part of this later, few studies have investigated their antimicrobial activities (Stassi et al., 1996; Angioni et al., 2003; Derwich et al., 2010).

The aim of this study was to identify the chemical composition of the oils of *J. phoenicea* and *C. sempervirens* and to compare the antimicrobial activity of them, in an attempt to contribute to the use of these as alternative products for microbial control and food preservation.

## MATERIALS AND METHODS

### Collection of plant materials

Collection information for the studied plant species is given below:

*J. phoenicea*: Sidi Safi-Tlemcen (Algeria), 21-11-2007

*C. sempervirens*: Maghnia-Tlemcen (Algeria), 13-11-2007

The botanical identification was achieved by Dr. Noury Benabadji, "Laboratoire d'Ecologie et Gestion des Ecosystèmes", Abou Bekr Belkaid University, Tlemcen (Algeria). Voucher specimens of the plants were deposited in the Herbarium of this laboratory.

### Isolation of the essential oils

The dried plant samples (leaves) were subjected to steam-distillation for 3 hours. Samples oils were dried over anhydrous sodium sulphate and stored at low temperature before analysis and bioassay.

### Gas chromatography-mass spectrometry

GC-MS analyses were carried out using a Hewlett-Packard 5890/5971A system fitted with a HP1 column (50 m x 0.20 mm fused silica capillary column; film thickness, 0.5 µm). GC oven initial temperature was 60°C and was programmed to 220°C at a rate of 2°C/min and 220°C during 120 min under the following operation conditions: vector gas, He; injector and detector temperatures, 250°C; injected volume: 0.2 µl, with a ratio split of 1/100. Retention indices were determined with C<sub>7</sub> - C<sub>28</sub> alkane standards as reference. The mass spectra were performed at 70 eV of the mass range of 35 - 400 amu. Identification of the constituents was based on comparison of the retention times with those of authentic samples and on computer matching against commercial (Wiley, MassFinder 2.1 Library, NIST98) and home-made libraries and MS literature data (McLafferty and Stauffer, 1989; Adams, 1995; Joulain and König, 1998; Joulain et al., 2001) and confirmed by

comparison of retention indices with published index data (ESO, 2000).

## Antimicrobial activity

### Microbial strains

The essential oils of *J. phoenicea* and *C. sempervirens* were individually tested against a panel of microorganisms. Five bacteria (reference strains) including 3 Gram-positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 11778) and 2 Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), and 3 fungi (*Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus stolonifer*) were used. The bacterial strains were supplied by Institute Pasteur, Algiers. The fungal strains were obtained from the Microbiology laboratory in Biology, Science Faculty of the Tlemcen University Algeria.

## Antibacterial screening

### Disc diffusion method

The disc diffusion method was employed for the determination of antibacterial activities of the essential oils in question (Lesueur et al., 2007). Paper discs (6 mm diameter; Sanofi Diagnostic Pasteur) were impregnated with 15 µl of the oil dissolved in DMSO (final concentration of 10% (v/v)) and transferred onto the Mueller-Hinton agar present in Petri dishes, which had been surface spread with 0.1 ml of bacterial suspension adjusted to 10<sup>7</sup> CFU/ml for *S. aureus* and 10<sup>6</sup> CFU/ml for the others strains (Joffin and Leyral, 2001; Pessini et al., 2003; Careaga et al., 2003). The DMSO solvent was used as the negative control. Standard antibiotics amoxicillin-clavulanic acid (30 µg/disk), tetracycline (30 µg/disk), trimethoprim-sulphamethoxazol (25 µg/disk) and cephalexin (30 µg/disk) were used as positive controls. After incubation at 37 ± 1°C during 24 h, the diameters of inhibition zones were measured in millimeters. Tests were carried out in triplicate.

## Determination of minimum inhibitory concentration (MIC)

The minimal inhibition concentration (MIC) values were determined for the bacterial strains to the essential oils of *J. phoenicea* and *C. sempervirens*. A 100 µl of the inoculum, initially adjusted to the density cited above, was spread onto 20 ml Mueller-Hinton agar supplemented with the oil at concentrations ranging from 2 - 10 µl/ml in Petri dishes, with each one its equivalent in DMSO. These serially cultures were then incubated at 37 ± 1°C for 24 h. The MIC is defined as the lowest concentration at which the microorganism does not demonstrate visible growth. As control, DMSO was used. Tests were carried out in triplicate.

## Antifungal screening

Antifungal activities of the essential oils were investigated by using a direct contact assay (Fandohan et al., 2004). Briefly, Five hundred microliters of the oil were mixed with 20 ml of the PDA (Potato dextrose agar) and were then put in the Petri dish (final concentration is 25 µl/ml). A disc (6 mm in diameter) of the fungal species was cut from (2 - 5)-days-old cultures on PDA plates and then the mycelia surface of the disc was placed upside down on the centre of a dish with fungal species in contact with growth medium on the dish. Then, the plates were incubated at 25 ± 2°C. PDA plates, without essential oils, were used as negative control. In

addition, PDA plates treated with amphotericin B (200 µg/ml) were used as positive control. The diameters of growth of the hyphae were measured after 48 h and 7 days, respectively, for *Rhizopus stolonifer* and for the others strains. All the experiments were replicated three times.

The percentage of growth inhibition by treatment (T) was calculated using the following of Ebbot (Motiejūnaitė and Peičulytė, 2004):

$$T = \frac{D_k - D_0}{D_k} \times 100$$

Where:  $D_k$  and  $D_0$  are the average of three replicates of hyphal extension (mm) of controls and of plates treated with essential oils.

## RESULTS AND DISCUSSION

### Chemical composition of the essential oils

The essential oils were obtained by steam-distillation from the leaves of *J. phoenicea* and *C. sempervirens* with yields (relative to dry weight material) of 0.52% (w/w) and 0.26% (w/w), respectively. Barrero et al. (2006), Achak et al. (2009) and Derwich et al. (2010) obtain a yield of 0.70, 0.94 and 1.62% (w/w) of Moroccan *J. phoenicea* leaves. Algerian *C. sempervirens* had a yield at least three times inferior than the Cameroonian (1%) (w/w) (Tapondjou et al., 2005).

The results are obtained by GC-MS analyses of the essential oils from *J. phoenicea* and *C. sempervirens* (Table 1). This table showed that 36 (96.9%) and 35 compounds (91.6%) were identified, respectively; compared to Moroccan and Tunisian oils, they present 45 (72%) and 31 (99%) compounds identified (Achak et al., 2008). The oils were predominantly composed of monoterpene hydrocarbons (72.9 - 75.7%), with  $\alpha$ -pinene as major constituent (34.5 - 60.5%).  $\beta$ -phellandrene (22.4%) and  $\alpha$ -Terpinyl acetate (14.7%) were the second most important constituents of the *J. phoenicea* oil. While cedrol (8.3%) was found to be the second most important constituent of the *C. sempervirens* oil.

A similar result was obtained by Barrero et al. (2006) and Achak et al. (2008 and 2009) in their study of Moroccan *J. phoenicea*. They find that the largest group of constituents in the essential oil is the monoterpenes (71.1%) with  $\alpha$ -pinene (45.5%) and 38.2 - 58% for Achak et al. (2009) as the major compound, but in Moroccan leaves essential oil,  $\delta$ -3-carene is the second most important constituent (Barero et al., 2006; Achak et al., 2008, 2009), with 13.0% (Barero et al., 2006) and 7.6% (Achak et al., 2009), which in the Algerian leaves oil did not exceed 4.7%. Also our results are in agreement with those of Rezzi et al. (2001) and Cavaleiro et al. (2001) when  $\alpha$ -pinene,  $\beta$ -phellandrene and  $\alpha$ -Terpinyl acetate are the main constituents of leaves essential oil obtained from Corsican and Portuguese *J. phoenicea*.

In the case of *C. sempervirens*, a similar result was found by Tognolini et al. (2006), when  $\alpha$ -pinene is the

major component of leaves essential oil, but it is presented in lower content (26.4%) compared with our study (60.5%). While,  $\alpha$ -pinene is the second and the third major component, respectively, in the previous researches (Sacchetti et al., 2005; Tapondjou et al., 2005).

### Antibacterial activity of the essential oils

The antibacterial activity of the essential oils was evaluated against five microorganisms, using disc diffusion and MIC methods. The disc diameters of zone of inhibition (DD), minimum inhibitory concentrations (MIC) of essential oils for the microorganisms tested (Table 2).

Results showed that the oils inhibited the growth of bacterial strains produced a zone diameter of inhibition from 6.8 - 15.6 mm, depended on susceptibility of the tested bacteria. However, the inhibition zones were lower than those of antibiotics, which showed wide inhibition zones at very low concentrations. As it can be seen in Table 2, *Enterococcus faecalis* was the most sensitive microorganism with the highest inhibition zone (15.6 mm) and lowest MIC value (7µl/ml) to the essential oil of *J. phoenicea*, whereas the oil of *C. sempervirens* was not active against this bacterial strain. On the other hand, it is seen from Table 2 that *Pseudomonas aeruginosa* was resistant at these essential oils.

### Antifungal activity of the essential oils

The essential oils isolated from the leaves of *J. phoenicea* and *C. sempervirens* were tested for antifungal activity against three fungal strains and their fungistatic effects were compared with the commercial antifungal amphotericin B. The percentages of growth inhibition (Table 3).

The results of antifungal activity assays showed that the oils moderately reduced the growth of *Aspergillus flavus* and *Fusarium oxysporum* (Table 3). However, the oils were significantly not active against *Rhizopus stolonifer*. Inhibitory effects of the oils on the growth of fungal strains were lower compared to amphotericin B. As seen from Table 3, the oils exhibited similar inhibition effects on the growth of tested fungi, which might be attributed to their similar compositions.

The antimicrobial activity of the essential oils of *J. phoenicea* and *C. sempervirens* could, in part, be associated with their major constituents such as  $\alpha$ -pinene,  $\beta$ -phellandrene,  $\alpha$ -Terpinyl acetate and cedrol. These components have been reported to display antimicrobial effects (Cosentino et al., 1999; Alessandra et al., 2005; Yang et al., 2007; Demirci et al., 2007). The essential oils containing terpenes are also reported to possess antimicrobial activity (Dorman and Deans, 2000), which are consistent with our present study. In

**Table 1.** Chemical composition of *J. phoenicea* and *C. sempervirens* essential oils.

Compound	RI <sup>a</sup>	(%)	
		<i>J. phoenicea</i>	<i>C. sempervirens</i>
Tricyclene	921	0.2	0.5
α-Thujene	925	0.1	0.4
α-Pinene	937	34.5	60.5
Camphene	948	0.5	0.5
Sabinene	971	0.7	1.3
β-Pinene	976	1.8	2.9
Myrcene	990	5.9	3.9
α-Phellandrene	999	0.6	-
δ-3-carene	1007	4.7	0.2
α-Terpinene	1016	0.2	0.2
p-Cymene	1023	-	0.2
Limonene	1025	1.2	4.6
β-Phellandrene	1034	22.4	-
γ-Terpinene	1057	0.1	0.5
Terpinolene	1085	1.9	2
Linalol	1099	0.1	tr
Pinocarveol	1140	-	tr
Borneol	1152	0.2	tr
α-Terpineol	1172	0.1	tr
Citronellol	1227	1.2	-
Thymol methyl ether	1228	-	0.2
Linalyl acetate	1248	0.3	tr
Bornyl acetate	1283	0.5	0.3
Carvacrol	1290	-	tr
Thymol	1298	-	tr
α-Terpinyl acetate	1349	14.7	-
β-Bourbonene	1381	tr	-
β-Elemene	1386	0.2	-
β-Caryophyllene	1417	1	0.3
γ-Elemene	1426	0.2	tr
α-Humulene	1452	0.4	0.3
Allo-aromadendrene	1459	-	0.5
γ-Muurolene	1472	0.1	0.2
Germacrene D	1478	1.5	2.3
β-Selinene	1486	tr	-
α-Muurolene	1496	0.2	0.2
γ-Cadinene	1510	0.1	0.1
δ-Cadinene	1516	0.5	0.6
Elemol	1546	0.4	-
Caryophyllene Oxide	1580	-	0.1
Cedrol	1607	-	8.3
β-Eudesmol	1630	tr	0.2
α-Eudesmol	1653	0.2	0.3
Manoyl Oxide	1988	0.2	-
Total identified (%)		96.9	91.6

<sup>a</sup> Compounds are listed in order of their elution from an HP1 column using the homologous series of n-alkanes.

<sup>b</sup> Trace (< 0.1%).

<sup>c</sup> Not detected.

**Table 2.** Antibacterial activities of the essential oils of *J. phoenicea* and *C. sempervirens*.

Bacterial strains	<i>J. phoenicea</i>		<i>C. sempervirens</i>		Standards antibiotics				Negative control
	DD	MIC	DD	MIC	SXT <sup>a</sup>	CL <sup>b</sup>	TE <sup>c</sup>	AMC <sup>d</sup>	
<i>Staphylococcus aureus</i> ATCC 25923	10.3	-	10.3	-	17	28	16	15	6.0
<i>Enterococcus faecalis</i> ATCC 29212	15.6	7	9.0	-	20	30	22	16	6.0
<i>Bacillus cereus</i> ATCC 11778	7.0	-	7.6	-	06	06	20	06	6.0
<i>Escherichia coli</i> ATCC 25922	9.6	-	9.3	-	17	40	42	07	6.0
<i>Pseudomonas aeruginosa</i> ATCC 27853	6.8	-	7.0	-	06	06	06	06	6.0

DD: diameter of inhibition zone (mm) including disc diameter of 6 mm, -: not active.

MIC: minimal inhibitory concentration (µl/ml).

<sup>a</sup>SXT: trimethoprim-sulphamethoxazol (25 µg/disk)

<sup>b</sup>CL: cephalixin (30 µg/disk)

<sup>c</sup>TE: tetracyclin (30 µg/disk)

<sup>d</sup>AMC: amoxicillin-clavulanic acid (30 µg/disk).

**Table 3.** Antifungal activities of the essential oils of *J. phoenicea* and *C. sempervirens*.

Fungal strains	<i>J. phoenicea</i>		<i>C. sempervirens</i>		AMP B <sup>a</sup>	Negative control
	G	I	G	I	I	G
<i>Aspergillus flavus</i>	35	40.6	36	39.0	88.0	59
<i>Fusarium oxysporum</i>	37	47.1	31	55.7	88.1	70
<i>Rhizopus stolonifer</i>	80	0.0	80	0.0	68.0	80

G: Growth [Colony diameter (mm)].

I: Inhibition [The percentage of growth inhibition (%)].

<sup>a</sup> AMP B: Amphotericin B (200µg/ml).

addition, the components in lower amount may also contribute to antimicrobial activity of the essential oils, involving probably some type of synergism with other active compounds (Marino et al., 2001).

## Conclusion

The results of this work show that the *J. phoenicea* and *C. sempervirens* essential oils possess antimicrobial properties, which can be used as natural antimicrobial agents for human and infectious diseases and in food preservation. Furthermore, the development of natural antimicrobial agents will help to decrease negative effects (pollution in environment, resistance) of synthetic chemicals and drugs.

## ACKNOWLEDGEMENT

The authors would like to thank Prof. Noury Benabadi, "Laboratoire d'Ecologie et Gestion des Ecosystèmes", Abou Bekr Belkaid University, Tlemcen (Algeria) for the identification of the plant materials, and Dr. Abdellah Moussaoui (Laboratoire des Produits Naturels) Abou Bekr Belkaid University, Tlemcen (Algeria) for providing us with the fungal strains.

## REFERENCES

- Adams RP (1995). Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publ. Corp., Carol Stream, IL, USA.
- Achak N, Romane A, Abbad A, Ennajar M, Romdhane M, Abderrabba A (2008). Essential oil composition of *Juniperus Phoenicea* from Morocco and Tunisia. J. Essent. Oil-Bearing Plants 1(2): 137-142.
- Achak N, Romane A, Alifriqui M, Adams RP (2009). Chemical studies of leaf essential oils of three species of *juniperus* from tensift al haouz-marrakech region (morocco). J. Essent. Oil Res. 21(4): 337-341.
- Alessandra LO, Roberta BL, Fernando AC, Marcos NE (2005). Volatile compounds from pitanga fruit (*Eugenia uniflora* L.). Food Chem. 99: 1-5.
- Allali H, Benmehdi H, Dib MA, Tabti B, Ghalem S, Benabadi N (2008). Phytotherapy of diabetes in West Algeria. Asian J. Chem. 20: 2701-2710
- Amer MMA, Wasif MM, Abo-Aytta AM (1994). Chemical and biological evaluation of *Juniperus phoenicea* as a hypoglycaemic agent. J. Agric. Res. 21: 1077-1091.
- Angioni A, Barra A, Maria T, Russo MT, Coroneo V, Sandro Dessia S, Cabras P (2003). Chemical composition of the essential oils of *Juniperus* from ripe and unripe berries and leaves and their antimicrobial activity. J. Agric. Food Chem. 51: 3073-3078.
- Barrero AF, Herrador MM, Arteaga P, Quilez del Moral JF, Sánchez-Fernández E (2006). Chemical Composition of the Essential Oil from the Leaves of *Juniperus phoenicea* L. from North Africa. J. Essent. Oil Res. 18: 168-169.
- Bellakhder J (1997). La Pharmacopée Marocaine traditionnelle. Paris: Ibis Press p. 272.
- Careaga M, Fernández E, Dorantes L, Mota L, Jaramillo ME, Hernandez-Sanchez H (2003). Antibacterial activity of *Capsicum* extract against *Salmonella typhimurium* and *Pseudomonas aeruginosa* inoculated in raw beef meat. Int. J. Food Microbiol.

- 83: 331-335.
- Cavaleiro C, Rezzi S, Salgueiro L, Bighelli A, Casanova J, Cunha AP (2001). Intraspecific chemical variability of the leaf essential oil of *Juniperus phoenicea* var. *turbinata* from Portugal. *Biochem. Systematics Ecol.* 29: 1175-1183.
- Chanegriha N, Baâliouamer A, Meklati BY, Favre-Bonvin J, Alamercury S (1993). Chemical composition of Algerian cypress essential oil. *J. Essent. Oil Res.* 5: 671-674.
- Chanegriha N, Baâliouamer A, Meklati BY, Chretien JR, Keravis G (1997). GC and GC/MS leaf oil analysis of four Algerian cypress species. *J. Essent. Oil Res.* 9: 555-559.
- Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E, Palmas F (1999). *In vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Lett. Appl. Microbiol.* 29: 130-135.
- Demirci B, Kosar M, Demirci F, Dinc M, Baser KHC (2007). Antimicrobial and antioxidant activities of the essential oil of *Chaerophyllum libanoticum* Boiss. et Kotschy. *Food Chem.* 105: 1512-1517.
- Derwich E, Benziane Z, Boukir A (2010). Chemical Composition of Leaf Essential Oil of *Juniperus phoenicea* and Evaluation of its Antimicrobial Activity. *Inter. J. Agri. Biol.* 12(2): 199-204.
- Dorman HJD, Deans SG (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* 88: 308-316.
- ESO (2000). The complete database of essential oils. Boelens Aroma Chemical Information Service, the Netherlands (1999).
- Fandohan P, Gbenou JD, Gnonlofin B (2004). Effet of essential oils on the growth of Fumonisin contamination in corn. *J. Agric. Food Chem.* 52: 6824-6829.
- Isman MB (2000). Plant essential oils for pest and disease management. *Crop Prot.* 19: 603-608.
- Joffin JN, Leyral G (2001). *Microbiologie technique 1 dictionnaire des techniques*. 3<sup>ème</sup> Ed. Biologie Technique p. 58.
- Joulain D, König WA (1998). The atlas of spectral data of sesquiterpene hydrocarbons. E.B.-Verlag, Hamburg.
- Joulain D, König WA, Hochmuth DH (2001). Terpenoids and related constituents of essential oils. Hamburg, Germany: Library of MassFinder 2.1.
- Larousse (2001). *Encyclopédie des plantes médicinales: Identification, préparations, soins*. Ed. Larousse, Londres pp. 14-29.
- Lesueur D, Rocca Serra de D, Bighelli A, Hoi TM, Ban NK, Thai TH, Casanova J (2007). Chemical composition and antibacterial activity of the essential oil of *Michelia faveolata* Meryll ex Dandy from Vietnam. *Flavour Fragr. J.* 22: 317-321.
- Lis-Balchin M, Deans SG (1997). Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. Appl. Bacteriol.* 82: 759-762.
- Marino M, Bersani C, Comi G (2001). Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *Int. J. Food Microbiol.* 67: 187-195.
- McLafferty FW, Stauffer DB (1989). *The Wiley/NBS Registry of Mass Spectral Data*. J. Wiley and Sons, New York.
- Misra G, Pavlovstathis SG (1997). Biodegradation kinetics of monoterpenes in liquid soil-slurry systems. *Appl. Microbiol. Biotechnol.* 7: 572-577.
- Motiejūnaitė O, Peičulytė D (2004). Fungicidal properties of *Pinus sylvestris* L. for improvement of air quality. *Medicina (Kaunas)* 40(8): 787-794.
- Mukherjee PK, Saritha GS, Suresh B (2002). Antimicrobial potential of two different *Hypericum* species available in India. *Phytother. Res.* 16: 692-695.
- Pessini GL, Prado Dias Filho Celso B, Nakamura V, Cortez DAG (2003). Antibacterial activity of extracts and neolignans from *Piper Regnellii* (Miq.) C. DC. Var. *pallescens* (C. DC.) yunk. *Memorias do Instituto Oswaldo Cruz* p. 98.
- Pierre-Leandri C, Fernandez X, Lizzani-Cuvelier L, Loiseau A-M, Fellous R, Garnerio J, Andréoli C (2003). Chemical composition of cypress essential oils: volatile constituents of leaf from seven cultivated *Cupressus* species. *J. Essent. Oil Res.* 15: 242-247.
- Reynolds JEF (1996). *Martindale-the Extra Pharmacopeia*, 31st ed. Royal Pharmaceutical Society of Great Britain, London.
- Rezzi S, Cavaleiro C, Bighelli A, Salgueiro L, Cunha AP, Casanova J (2001). Intraspecific chemical variability of the leaf essential oil of *Juniperus phoenicea* subsp. *turbinata* from Corsica. *Biochem. Systematics Ecol.* 29: 179-188.
- Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem.* 91: 621-632.
- Service RF (1995). Antibiotics that resist resistance. *Science* 270: 724-727.
- Stassi V, Vervokidou E, Loukis A, Harvala C, Philianos S (1996). The antimicrobial activity of the essential oils of four *Juniperus* species growing wild in Greece. *Flav. Fragr. J.* 11: 71-74.
- Tapondjou AL, Adler C, Fontem DA, Bouda H, Reichmuth C (2005). Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. *J. Stored Prod. Res.* 41: 91-102.
- Tognolini M, Barocelli E, Ballabeni V, Bruni R, Bianchi A, Chiavarini M, Impicciatore M (2006). Comparative screening of plant essential oils: Phenylpropanoid moiety as basic core for antiplatelet activity. *Life Sci.* 78: 1419-1432.
- Yang JK, Choi MS, Seo WT, Rinker DL, Han SW, Cheong GW (2007). Chemical composition and antimicrobial activity of *Chamaecyparis obtusa* leaf essential oil. *Fitoterapia* 78: 149-152.
- Zavarin E, Lawrence L, Thomas MC (1971). Chemosystematics of *Cupressus*. IV. Compositional variations of leaf monoterpenes in *Cupressus macrocarpa*, *C. pygmaea*, *C. goveniana*, *C. abramsiana* and *C. sargentii*. *Phytochem.* 10: 379-339.