Azzeddine Zeraib 1,2
Messaoud Ramdani 2
Lamia Boudjedjou 2
Pierre Chalard 3
Gilles Figuredo 4

Authors’ addresses:
1 Department of Molecular Biology, Abbes Laghrour University, 40000 Khencela, Algeria.
2 Laboratory of Natural Resource Valorization, Ferhat Abbas University, 19000 Setif, Algeria.
3 Blaise Pascal University, BP 10448, F-63000 Clermont Ferrand, France.
4 LEXVA Analytique, 63110 Beaumont, France.

Correspondence: Azzeddine Zeraib
Department of Biology Molecular, Abbes Laghrour University, 40000 Khencela, Algeria.
Tel.: +213 778 168 697
e-mail: azzeraib@yahoo.fr

Article info:
Received: 5 April 2014
Accepted: 9 June 2014

RESEARCH ARTICLE

Chemical composition and antibacterial activity of Juniperus thurifera L. essential oils

ABSTRACT

The qualitative and quantitative composition of the essential oils obtained from male and female leaves of Juniperus thurifera L.; (growing in Algeria) has been investigated for the first time. The essential oils were obtained by hydrodistillation (0.45% from female trees and 0.53% from male trees, v/w dried material) and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Seventy-seven compounds were identified, representing more than 97% of the oils. The major components were Sabinen, α-pinene and terpinene-4-ol. The concentrations of the oil constituents: α-thujene, α-pinene, α-phellandrene, p-cymene, linalyl acetate, Δ-amorphene, germacrene D-4-ol, and 4-epi-abietal were greater in the oil of the female tree than in the oil of the male tree. Conversely, the concentrations of α-terpineol, γ-terpinene, terpinene-4-ol, elemol, α-epi-cadinol and α-eudesmol were greater in the oil of the male tree than in the oil of the female tree. However, the concentration gradient trends for both female and male trees were similar for sabinen, myrcene, linalool, β-pinene, limonene, cis-sabinene hydrate terpinolene, α-terpineol. The antimicrobial activity of male and female J. thurifera essential oils was evaluated against 14 bacteria. The results showed a variable degree of antibacterial activity depending from the type of the oil (extracted from male or female trees). Essential oils of female trees were most effective.

Key words: Cupressaceae, Juniperus thurifera L., sex of the trees, essential oil, antimicrobial activity

Introduction

The genus Juniperus L. (Cupressaceae) contains more than 67 species. It is quite widespread in the northern hemisphere, although J. procera Hochst. ex Endl. also grows southward along the rift mountains in East Africa in the southern hemisphere. The genus Juniperus is divided into three sections: Caryocedrus (one species, J. drupacea Labill.); Juniperus (*Oxycedrus, 9 or 10 species) and Sabina (the remaining, approximately 50 species) (Adams & Demeke, 1993; Adams, 1998, 2000, 2008). The flora of Algeria lists two sections and five Juniperus species; Sect. Oxycedrus (J. communis L., J. oxycedrus L.), and Sabina (J. thurifera L., J. phonicea L., J. sabina L.) (Quezel & Sainta, 1963; Maire, 1967; Adams et al., 2003). All over the world plants from this genus have always been regarded as a well-known traditional remedy due to their numerous therapeutic properties, such as anti-inflammatory, diuretic, antiseptic (bacterial and fungal), hypoglycaemic, hypotensive, analgesic and abortifacient (Stassi et al., 1996; Milos & Radonic, 2000; Lesjak et al., 2011; Öztürk et al., 2011).

Thuriferous juniper (Juniperus thurifera L.) grows in the western part of the Mediterranean basin (France, Spain, Italia, Algeria and Morocco). It is a dioecious tree or shrub, with scale leaves and bluish black berries at maturity (Gauquelin, 2003). African subpopulations of Juniperus thurifera have been described as distinct species (Juniperus africana (Maire) Villar or more recently as a subspecies (J. thurifera
subsp. africana (Maire) Romo and Boratynski (Romo & Boratynski, 2007). Genetic analysis also indicates that the Moroccan and Algerian subpopulations are genetically distinct and have been isolated from other subpopulations for several millennia (Terrab et al., 2008).

Gender-related differences in growth and concentration of secondary metabolites have been documented in dioecious plants. Males usually grow faster than females, whilst females allocate more resources to reproduction and chemical defenses than males, hence their growth is reduced (McGowan et al., 2004; Massei et al., 2006; Cepeda-Cornejo & Dirzo, 2010). These physiological differences could also affect the secondary metabolism, as the production of volatile compounds (Gobbo-Neto & Lopes, 2007). In fact, a variation in the essential oil composition and phenolic component from male and female specimens was previously observed (Elmqvist et al. 1991; Riddle et al. 1996; Koztowska et al., 2005; Massei et al., 2006; Afsharypuor et al., 2007; Lago et al., 2008; Eiter et al., 2010; Iszkulo et al., 2011).

In recent years, essential oils and plant extracts have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds, such as antibacterial, antifungal and insecticidal substances (Celiktas et al., 2007; Hammami et al., 2011).

The hypothesis of this study was that the essential oil content and composition may be different in male and in female trees. Several works studied the chemical composition of the essential oils of Juniperus thurifera L. and their antimicrobials activity in different regions of the Mediterranean basin as France, Spain, the Pyrenees, the Corsican and to Morocco (Hernandez et al., 1987; Adams, 1999; Adams et al., 2003; Barrero et al., 2004; Achak et al., 2008; Achak et al., 2009; Mansouri et al., 2010, Bahri et al., 2013). But any work has not reported the effect of the sex of the trees on chemical composition of essential oils of Juniperus thurifera L.

Therefore, the objective of this study was to evaluate the variations in the yields, essential oil composition and their antibacterial activity within single male and female trees of Algerian thuriferous juniper (Juniperus thurifera L.).

Materials and Methods

Plant material

The leaves of male and female trees of J. thurifera, were collected from Aures Mountains (Algeria), at 1550 m of altitude. A voucher specimen of each plant (sexes) is deposited in the herbarium of the Laboratory of Natural Resource Valorization, Faculty of Biology, Farhat Abbes University, Setif, Algeria.

The essential oils were extracted by hydrodistillation using Clevenger-type apparatus for three hours according to Tumen et al. (2010). The prepared volatile oils were dehydrated over anhydrous sodium sulphate and stored in sealed glass vials at 4-5°C prior to analysis.

Essential oil analysis

The essential oil analysis is performed on a chromatography's type Hewlett-Packard HP 7890 equipped with a capillary column DB-5 (length: 30 m, and 0.25 mm internal diameter, film thickness is 0.25 mm) coupled to a mass spectrometer (MS) type with a Hewlett Packard 5975 detector impact of electrons, 70 EV. The analytical conditions are as follows: injector temperature: 250°C, detector temperature: 280°C oven programming: 50 ° C for five min, 5°C / min from 50° to 300°C and isothermal 300°C for five min. The carrier gas is helium at a rate of one ml/min. Injector split mode 1:100. The GC analysis was carried out using an Agilent 6890N GC system equipped with a FID detector operated at a temperature of 300°C. To obtain the same elution order of peaks detected by GC/MS, simultaneous injection on the GC was performed using the same column and appropriate chromatographic conditions as those described for the GC/MS system.

Identification of the essential oil components was carried out by comparing their mass spectra and their KI (Kovats index) with those databases (Adams, 2001) and that established by the laboratory. The relative concentrations of the separated compounds based on percentage were computed from chromatograms obtained with the GC/FID system.

Antibacterial activity

Gram positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus aureus clinical and methicillin-resistant Staphylococcus aureus) and Gram negative bacteria (Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922, Escherichia coli clinical, Serratia marcescens, Enterobacter cloacae, Proteus permeri, Acinetobacter baumannii, Salmonella para, Salmonella spong, Shigella sp. and Meningo sp.) were used in this study.

The antimicrobial activity was tested by the agar-well diffusion method according to Mighri et al. (2010) with slight
were most abundant, 63.2% in female trees and 58.53% in male trees. The main monoterpenes hydrocarbons were sabinene (32.11% in female trees, 31.16% in male trees) and α-pinene (13.04% in female trees, 7.84% in male trees), while the main oxygenated monoterpenes were terpinen-4-ol (4.91% in female trees, 11.65% in male trees). Diterpene hydrocarbons were present in smaller amounts, 0.28% and 1.31%, oxygenated Diterpenes, 0.46% and 1.51%, and sesquiterpene hydrocarbons, 1.61% and 2.91% for essential oil of male and female trees respectively. Oxygenated sesquiterpenes were present in a high percentage in essential oil of male trees, amounting to 15.26% of the total essential oil content, while the essential oils of female trees contain 11.9% of the total essential oil content, with elemol as the main compound, 8.28% and 3.27% for essential oil of male and female trees respectively.

The concentrations of the oil constituents: α-thujene, α-pinene, α-phellandrene, p-cymene, linalyl acetate, Δ-amorphene, germacrene D-4-ol, and 4-epi-abietal were greater in the oil of the female tree than in the oil of the male tree. Conversely, the concentrations of α-terpinene, γ-terpinene, terpinene-4-ol, elemol, α-epi-cadinol and α-eudesmol were greater in the oil of the male tree than in the oil of the female tree. However, the concentration gradient trends for both female and male trees were similar for sabinene, myrcene, linalool, β-pinene, limonene, cis-sabinene hydrate terpinolene, α-terpinol.

Certain components detected in the female tree were not detected in the male trees, including β-phellandrene, isobutyl benzene, α-murolène, epoxyme humulene II, valencene, α-muurolol, β-eudesmol and phytol. While the components, δ-2-carene, p-cymene-7-ol, verbanone, piperitone, cedrol, 1-epi-cubenol, cubenol and γ-gurjunene, were detected in the male tree and were not detected in the female trees.

Frequently, in dioecious plants, female plants allocate more resources to reproduction than male plants. Therefore it is expected that asymmetrical allocation to reproduction may lead to a reproduction-growth tradeoff, whereby female plants grow less than male plants, but invest more in defenses and thus experience lower herbivory than male plants (Cepeda-Cornejo & Dirzo, 2010). Typically, males allocate more resources to growth and females to high concentrations of secondary metabolites. Contrary to predictions, in this study the males had also higher yield in essential oils than females. This result is similar to that obtained by Asili et al. (2008a, 2010) for Juniperus communis subsp. hemisphaerica, Juniperus oblonga and Juniperus foetidissima, and
Zheljazkov et al. (2013) for Juniperus scopulorum Sarg. The yield in essential oils obtained from the female leaves of Juniperus sabina, and J. excelsa subsp. polycarpos is higher to that obtained from male leaves (Asili et al., 2008b, 2010).

The results of the present study confirmed the prevalence of monoterpenoid hydrocarbons compared to other components. There are few previous reports on the phytochemical studies of the male and female essential oil of Juniperus species growing in other parts of the world indicated this results. Monoterpene hydrocarbons represented the most abundant constituents of the oil of both male and female leaves of shrubs of J. communis subsp. hemisphaerica (60.2 and 60.6% respectively, J. oblonga, (57.4 and 63.6% respectively) (Asili et al., 2008a), J. excelsa subsp. polycarpos (81.57 and 66.42 % respectively) (Asili et al., 2008b; Emami et al., 2011), J. chinensis L. (60.2 and 60.6% respectively, J. oblonga, (37.2 and 42.7% respectively) (Afsharypuor et al., 2007), J. sabina (63.8 and 62.2% respectively) and J. foetidissima (75.9 and 68.3% respectively) (Asili et al., 2010).

A correlation between the chemical composition of the essential oil and the sex of the tree has been observed in several species of the genus Juniperus L. (Riddle et al., 1996; Afsharypuor et al., 2007; Zheljazkov et al., 2013) and are well known for other family (Lago et al., 2008; Eiter et al., 2010; Gourine et al., 2010; Iszkuło et al., 2011).

Table 1. Chemical composition of male and female J. thurifera essential oils.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ki</th>
<th>Male (%)</th>
<th>Female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-thujene</td>
<td>924</td>
<td>1.8</td>
<td>2.06</td>
</tr>
<tr>
<td>α-pinene</td>
<td>932</td>
<td>7.86</td>
<td>13.04</td>
</tr>
<tr>
<td>fenchene</td>
<td>946</td>
<td>0.03</td>
<td>0.1</td>
</tr>
<tr>
<td>camphene</td>
<td>947</td>
<td>0.1</td>
<td>0.15</td>
</tr>
<tr>
<td>sabinene</td>
<td>973</td>
<td>31.16</td>
<td>32.11</td>
</tr>
<tr>
<td>β-pinene</td>
<td>976</td>
<td>1.18</td>
<td>1.56</td>
</tr>
<tr>
<td>myrcene</td>
<td>988</td>
<td>3.36</td>
<td>3.26</td>
</tr>
<tr>
<td>δ-2-carene</td>
<td>996</td>
<td>0.13</td>
<td>-</td>
</tr>
<tr>
<td>α-phellandrene</td>
<td>1006</td>
<td>0.06</td>
<td>1.06</td>
</tr>
<tr>
<td>α-terpinene</td>
<td>1015</td>
<td>2.72</td>
<td>1.47</td>
</tr>
<tr>
<td>p-cymene</td>
<td>1022</td>
<td>0.22</td>
<td>1.13</td>
</tr>
<tr>
<td>limonene</td>
<td>1027</td>
<td>1.7</td>
<td>1.1</td>
</tr>
<tr>
<td>β-phellandrene</td>
<td>1028</td>
<td>-</td>
<td>0.33</td>
</tr>
<tr>
<td>(Z)-β-ocimene</td>
<td>1035</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>(E)-β-ocimene</td>
<td>1045</td>
<td>0.21</td>
<td>0.4</td>
</tr>
<tr>
<td>γ-terpinene</td>
<td>1057</td>
<td>4.09</td>
<td>2.24</td>
</tr>
<tr>
<td>cis-sabinene hydrate</td>
<td>1069</td>
<td>1.97</td>
<td>1.51</td>
</tr>
<tr>
<td>terpinolene</td>
<td>1083</td>
<td>1.77</td>
<td>1.3</td>
</tr>
<tr>
<td>p-cymenene</td>
<td>1088</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>linalool</td>
<td>1098</td>
<td>3.14</td>
<td>3.77</td>
</tr>
<tr>
<td>cis-thujone</td>
<td>1104</td>
<td>0.17</td>
<td>0.24</td>
</tr>
<tr>
<td>trans-thujone</td>
<td>1114</td>
<td>0.17</td>
<td>0.55</td>
</tr>
<tr>
<td>cis-p-menth-2-en-1-ol</td>
<td>1123</td>
<td>0.85</td>
<td>0.38</td>
</tr>
<tr>
<td>α-comphlenal</td>
<td>1129</td>
<td>0.07</td>
<td>0.22</td>
</tr>
<tr>
<td>trans-p-menth-2-en-1-ol</td>
<td>1142</td>
<td>0.52</td>
<td>0.2</td>
</tr>
<tr>
<td>camphor</td>
<td>1144</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>E-tagetone</td>
<td>1147</td>
<td>0.1</td>
<td>0.18</td>
</tr>
<tr>
<td>terpinene-4-ol</td>
<td>1181</td>
<td>11.65</td>
<td>4.91</td>
</tr>
<tr>
<td>p-cymene-7-ol</td>
<td>1187</td>
<td>0.12</td>
<td>-</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>1195</td>
<td>1.23</td>
<td>1</td>
</tr>
<tr>
<td>verbanone</td>
<td>1205</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Trans-piperitol</td>
<td>1209</td>
<td>0.31</td>
<td>0.2</td>
</tr>
<tr>
<td>nerol 1 (80)</td>
<td>1224</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>Compound</td>
<td>Ki</td>
<td>Male (%)</td>
<td>Female (%)</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>linalyl acetate</td>
<td>1250</td>
<td>1.32</td>
<td>3.16</td>
</tr>
<tr>
<td>piperitone</td>
<td>1255</td>
<td>0.39</td>
<td>-</td>
</tr>
<tr>
<td>pregeijerene</td>
<td>1280</td>
<td>0.59</td>
<td>0.14</td>
</tr>
<tr>
<td>bronyl acetate</td>
<td>1284</td>
<td>0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>isobutyl benzene</td>
<td>1287</td>
<td>-</td>
<td>0.07</td>
</tr>
<tr>
<td>2,4-decadien-1-ol</td>
<td>1312</td>
<td>0.45</td>
<td>0.15</td>
</tr>
<tr>
<td>terpenyl acetate</td>
<td>1350</td>
<td>0.48</td>
<td>0.58</td>
</tr>
<tr>
<td>geranyl isobutyrate</td>
<td>1357</td>
<td>0.11</td>
<td>0.28</td>
</tr>
<tr>
<td>geranyl acetate</td>
<td>1377</td>
<td>0.21</td>
<td>0.63</td>
</tr>
<tr>
<td>β-caryophyllene</td>
<td>1418</td>
<td>0.04</td>
<td>0.1</td>
</tr>
<tr>
<td>α-humulene</td>
<td>1457</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>germacrene-D</td>
<td>1482</td>
<td>0.11</td>
<td>0.21</td>
</tr>
<tr>
<td>α-muurolene</td>
<td>1497</td>
<td>-</td>
<td>0.36</td>
</tr>
<tr>
<td>γ-cadinene</td>
<td>1515</td>
<td>0.07</td>
<td>0.12</td>
</tr>
<tr>
<td>Δ-amanophene</td>
<td>1517</td>
<td>0.1</td>
<td>1.6</td>
</tr>
<tr>
<td>elemol</td>
<td>1550</td>
<td>8.28</td>
<td>3.27</td>
</tr>
<tr>
<td>(E)-nerolidol</td>
<td>1560</td>
<td>0.3</td>
<td>0.33</td>
</tr>
<tr>
<td>germacrene D-4-ol</td>
<td>1579</td>
<td>0.29</td>
<td>1.24</td>
</tr>
<tr>
<td>Oxyde de Caryophyllene</td>
<td>1585</td>
<td>0.49</td>
<td>0.83</td>
</tr>
<tr>
<td>Aromadendr-9-ene</td>
<td>1592</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>cedrol</td>
<td>1596</td>
<td>0.16</td>
<td>-</td>
</tr>
<tr>
<td>humulene epoxyde II</td>
<td>1605</td>
<td>-</td>
<td>0.7</td>
</tr>
<tr>
<td>epi-cedrol</td>
<td>1613</td>
<td>0.2</td>
<td>0.73</td>
</tr>
<tr>
<td>valencene</td>
<td>1618</td>
<td>-</td>
<td>0.15</td>
</tr>
<tr>
<td>1-epi-cubenol</td>
<td>1627</td>
<td>0.36</td>
<td>-</td>
</tr>
<tr>
<td>γ-eudesmol</td>
<td>1630</td>
<td>0.26</td>
<td>0.98</td>
</tr>
<tr>
<td>α-epi-cadinol</td>
<td>1635</td>
<td>1.23</td>
<td>0.31</td>
</tr>
<tr>
<td>cubenol</td>
<td>1643</td>
<td>0.26</td>
<td>-</td>
</tr>
<tr>
<td>α-muurolol</td>
<td>1646</td>
<td>-</td>
<td>0.42</td>
</tr>
<tr>
<td>β-eudesmol</td>
<td>1649</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>α-eudesmol</td>
<td>1659</td>
<td>2.74</td>
<td>1.76</td>
</tr>
<tr>
<td>2,6-Dimethyl-1,5-heptadien-4-ol acetate</td>
<td>1666</td>
<td>0.17</td>
<td>0.58</td>
</tr>
<tr>
<td>gurjunene</td>
<td>1668</td>
<td>0.24</td>
<td>0.15</td>
</tr>
<tr>
<td>γ-gurjunene</td>
<td>1678</td>
<td>0.42</td>
<td>-</td>
</tr>
<tr>
<td>epi-α-bisabolol</td>
<td>1686</td>
<td>0.3</td>
<td>0.22</td>
</tr>
<tr>
<td>2-pentadecanone</td>
<td>1698</td>
<td>0.33</td>
<td>0.1</td>
</tr>
<tr>
<td>(Z,Z)-farnésol</td>
<td>1714</td>
<td>0.09</td>
<td>0.36</td>
</tr>
<tr>
<td>Cyclohexene, 1,5,5-trimethyl-6-(2-propenylidene)</td>
<td>1780</td>
<td>0.52</td>
<td>0.42</td>
</tr>
<tr>
<td>sandaracopimara-8(14), 15-diene</td>
<td>1962</td>
<td>0.13</td>
<td>0.59</td>
</tr>
<tr>
<td>manoyl oxide</td>
<td>1989</td>
<td>0.05</td>
<td>0.28</td>
</tr>
<tr>
<td>Abietatriène</td>
<td>2057</td>
<td>0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>Abietatriène</td>
<td>2090</td>
<td>0.1</td>
<td>0.45</td>
</tr>
<tr>
<td>phytol</td>
<td>2109</td>
<td>-</td>
<td>0.14</td>
</tr>
<tr>
<td>4-epi-abietal</td>
<td>2299</td>
<td>0.41</td>
<td>1.27</td>
</tr>
<tr>
<td>Monoterpenes hydrocarbons</td>
<td></td>
<td>58.53</td>
<td>63.2</td>
</tr>
<tr>
<td>Oxygenated monoterpenes</td>
<td></td>
<td>21.57</td>
<td>16.84</td>
</tr>
<tr>
<td>Sesquiterpene hydrocarbons</td>
<td></td>
<td>1.61</td>
<td>2.91</td>
</tr>
<tr>
<td>Oxygenated sesquiterpenes</td>
<td></td>
<td>15.26</td>
<td>11.9</td>
</tr>
<tr>
<td>Diterpene hydrocarbons</td>
<td></td>
<td>0.28</td>
<td>1.31</td>
</tr>
<tr>
<td>Oxygenated Diterpenes</td>
<td></td>
<td>0.46</td>
<td>1.55</td>
</tr>
</tbody>
</table>
Antibacterial activity

The antibacterial activity of both essential oils was evaluated by paper disc diffusion method against 14 bacteria. A zone of inhibition around the discs shows a bactericidal or bacteriostatic activity, while the absence of inhibition zone proved no effect of the tested oils against the microorganisms. We consider that essential oil has bacteriostatic action if the diameter of inhibition is >12 mm (Sağdaç, 2003). The results of the inhibition trials are reported in Table 2. The results showed that the oils inhibited the growth of bacterial strains produced a zone diameter of inhibition from 6 mm (no inhibition) to 17 mm. The maximum zone of inhibition was recorded against Staphylococcus aureus ATCC 25923 (17 mm) and Escherichia coli ATCC 25922 (16 mm). On the other hand, the oil was ineffective against Pseudomonas aeruginosa ATCC 27853.

The variance analyses showed significant difference among the results from the antibacterial assays (Table 3). The zone diameter of inhibition depended on susceptibility of the tested bacteria, Escherichia coli ATCC 25922, Escherichia coli (clinical), Staphylococcus aureus ATCC 25923, Staphylococcus aureus (clinical), Salmonella para, Salmonella spomb, MRSA, are very sensitive again the essential oils, conversely, Meningo sp. Serratia marcescens, Enterobacter cloacae and Proteus permeri are not sensitive. Shigella sp. seems sensitive to the essential oils of J. thurifera. It is revealed that the Escherichia coli and Staphylococcus aureus was very sensitive to the essential oil of Moroccan J. thurifera, and two Pseudomonas strains proved resistant (Bahri et al., 2013).

The zone diameter of inhibition depended also on the essential oils type (from male or female trees). The essential oils of female trees showed a broad spectrum of antibacterial activity against Gram positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus aureus clinical and MRSA) and Gram negative bacteria (Escherichia coli clinical, Serratia marcescens, Proteus permeri, Meningo sp. and Shigella sp).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>EO male leaves</th>
<th>Gent.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>means ± sd</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>10.33±1.52</td>
<td>16±1</td>
</tr>
<tr>
<td>Staphylococcus aureus (clinical)</td>
<td>12.66±1.15</td>
<td>13.33±1.15</td>
</tr>
<tr>
<td>MRSA</td>
<td>10.33±0.57</td>
<td>13.66±1.52</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>15.66±0.57</td>
<td>7±0.57</td>
</tr>
<tr>
<td>Escherichia coli (clinical)</td>
<td>0.00</td>
<td>11.33±0.57</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>0.00</td>
<td>10.33±0.57</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>0.00</td>
<td>8.33±0.57</td>
</tr>
<tr>
<td>Proteus permeri</td>
<td>0.00</td>
<td>11.33±1.54</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>0.00</td>
<td>9.66±0.57</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Salmonella para</td>
<td>12.66±0.57</td>
<td>9.66±0.57</td>
</tr>
<tr>
<td>Salmonella spomb</td>
<td>13.66±0.57</td>
<td>11±0.57</td>
</tr>
<tr>
<td>Meningo sp.</td>
<td>0.00</td>
<td>10.66±0.57</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>8.33±0.57</td>
<td>10±1</td>
</tr>
</tbody>
</table>

MRSA = Methicillin-resistant Staphylococcus aureus; Gent. = Gentamicine; EO = Essential oil; sd = standard deviation ;(−) = not sensitive; (+)= sensitive; (+++) = very sensitive; (++++) = extremely sensitive.

| Table 3. Variance analysis of two factors controlled. |
|----------|----------|----------|----------|----------|
|          | dl       | SC       | CM       | F        | P        |
| Strains  | 13       | 476,488  | 36,653   | 58,09***| 0,000    |
| Essential oils | 1       | 63,440   | 63,440   | 100,55***| 0,000    |
| Interaction | 13       | 337,726  | 25,979   | 41,17***| 0,000    |
| Error    | 56       | 35,333   | 0,631    |          |          |
| Total    | 83       | 912,988  |          |          |          |
Conversely, the essential oils of male trees showed a broad spectrum of antibacterial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus*, *Salmonella para*, and *Salmonella spombre*. This variability in the antibacterial activity of male and female *J. thurifera* essential oils is due to their different chemical composition.

The inhibitory action of the essential oil could be attributed to the occurrence of high proportions of monoterpens and sesquiterpenes in the oil (Cakir et al., 2004). Antimicrobial properties of action might be related to these compounds which have a high potential in strongly inhibiting microorganism pathogens. The following components present are believed to play an important role as antibacterial agents, linalool, α-cadinol, globulol and viridiflorol, pulegone, α-pinene, terpinen-4-ol, germacrene A and para-methyl anisole corresponding to the amounts present in the essential oil (Hammami et al., 2011).

**Conclusion**

Analysis of the chemical composition of the essential oil of male and female *Juniperus thurifera* leaves has allowed identifying 77 compounds. The monoterpens represented the main portion of the oils (≈80% of the total essential oils). The majority compounds are the sabinene, α-pinene, terpinene-4-ol, elemol, γ-terpinene and linalool. The essential oil was found to be active against all the bacterial strains except the *Pseudomonas aeruginosa* ATCC 27853.

The sex of the tree affected the chemical composition of essential oil and their antibacterial activity. Female trees grow less than male trees, but invest more in defenses than male trees. Consequently, the essential oil of female trees had the best bactericidal activity than the essential oils of female trees.

**Acknowledgement**

This study was supported in part by the Chemistry of heterocyclic compounds and carbohydrates Laboratory, Higher National School of Chemistry, Clermont Ferrand (France) and the Ministry of Higher Education and Scientific Research of the Algerian People's Democratic Republic.

**References**


