



Research Article

# Chemical Composition and Antibacterial Activity of Berries Essential Oil of Algerian *Juniperus thurifera* (Var. *aurasiaca*)

Lamia Boudjedjou<sup>1,2</sup>, Messaoud Ramdani<sup>1</sup>, Azzeddine Zeraib<sup>3,4\*</sup>, Tarek Benmeddour<sup>2,3</sup>, Azzeddine Fercha<sup>4</sup>

<sup>1</sup>Laboratory of Natural Resources Valorization, Setif 1 University, 19000 Setif, Algeria.

<sup>2</sup>Department of Life and Natural Sciences, Mohamed Khider University, 7000 Biskra, Algeria.

<sup>3</sup>Laboratory of Genetics, Biotechnology and Valorization of Bio-resources, Mohamed Khider University, 7000 Biskra, Algeria.

<sup>4</sup>Department of Biology, Abbes Laghrour University, 40000 Khenchela, Algeria.

## Article Info

### Article History:

Received: 13 April 2018

Revised: 25 May 2018

Accepted: 30 May 2018

ePublished: 23 September 2018

### Keywords:

-Juniper  
-Essential oil  
-Antibiotics  
-Combination  
-Antibacterial activity

## ABSTRACT

**Background:** Over the past decade, most antibiotic research programs have focused on finding new compounds with antimicrobial activity. This study aims to investigate the chemical composition and antibacterial activity of the essential oil (EO) extracted from ripe berries of Algerian *Juniperus thurifera* var. *aurasiaca*.

**Methods:** The chemical composition of *J. thurifera* EO extracted by hydrodistillation was analyzed by using the GC-MS technique. Antibacterial activity of EO alone and in combination with three conventional antibiotics was assessed by using disc diffusion method against four bacterial strains.

**Results:** Thirty-five components were identified, representing ~87 % of the oil. The main components were m-mentha-6,8-diene (15.43 %),  $\beta$ -pinene (10.59 %), elemol (8.31 %) and terpinene-4-ol (7.44 %). The essential oil showed strong antibacterial activity against *S. aureus* and *E. coli*, but no activity against *P. aeruginosa* and *B. subtilis*. Synergistic effects were observed because of the combined application of EO with gentamicin against all strains tested, and with amoxicillin against *B. subtilis*. Furthermore, the combination of EO/cefazolin demonstrated an additive effect against *B. subtilis*. In contrast, the combination of EO with amoxicillin and cefazoline revealed antagonistic effects against *S. aureus*, *E. coli*, and *P. aeruginosa*.

**Conclusion:** This is the first report on the chemical composition and antibacterial activity of Algerian juniper berries' essential oil. The results indicate that the studied EO may be a promising source of antibacterial compounds that could be useful for pharmaceutical applications especially in combination with conventional antibiotics.

## Introduction

Nowadays, the rise of bacterial resistance to antibiotics has become a serious public health concern, highlighting the urgent need for new and readily available drugs for novel therapeutic options in both human and veterinary medicines.<sup>1</sup> The use of medicinal and aromatic plants as a source of antimicrobial drugs (e.g., essential oils) is appropriate because plants naturally produce a wide variety of secondary metabolites that can serve an important defensive role against bacteria, viruses, and other microbes. Therefore, the ineffectiveness of conventional therapy may be avoided by combining essential oils (EOs) with antibiotics (ABs).<sup>2</sup> In recent years, interest in the application of EOs in the treatment of infectious diseases has increased,<sup>3</sup> because they appear to exert synergistic interactions with ABs,<sup>4-8</sup> which reduces the minimum effective dose of ABs and prevents their side effects.<sup>9</sup>

*Juniperus thurifera* L. (*Cupressaceae*) is a medicinal plant used in folk medicine in Algeria and many countries

for the treatment of a variety of diseases.<sup>10,11</sup> This species is a dioecious tree or shrub endemic to the south-western Europe and Northern Africa.<sup>11,12</sup> The Algerian population of juniper (var. *aurasiaca*) is genetically closely related to the European population (subsp. *thurifera*), but is phytochemically and morphologically related to the Moroccan population (var. *africana*).<sup>10,13</sup> Although the chemical composition and biological properties of *J. thurifera* EOs are well documented,<sup>10-22</sup> the chemical composition of EO extracted from *J. thurifera* berries var. *hispanica* has been reported in only one study.<sup>14</sup> Furthermore, among the few studies that have dealt with the Algerian *J. thurifera* var. *aurasiaca*, none were conducted on berries EOs neither for their chemical composition nor their biological activities. Therefore, this study, for the first time, investigated the chemical composition and antibacterial activity of the EO extracted from berries of Algerian *J. thurifera* var. *aurasiaca*. Moreover, since the interaction of EOs with ABs is one of the new ways to overcome bacterial resistance,<sup>4-7</sup> it was

\*Corresponding Author: Zeraib Azzeddine, E-mail: [azzeraib@yahoo.fr](mailto:azzeraib@yahoo.fr)

©2018 The Authors. This is an open access article and applies the Creative Commons Attribution (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers.

also decided to test the potential interactions between essential oil of Algerian *J. thurifera* berries and conventional antibiotics.

## Materials and Methods

### Plant material and essential oil extraction

Samples of ripe berries were collected randomly from individual trees (~ 100 m apart from each other) in late Autumn (2015) in Ain El-baidha's mountain (1500 m of altitude) province of Batna, at 35.632°N of latitude and 6.213°E of longitude. The samples were then crushed and homogenized with a mortar and pestle. The species was identified by Dr. Zeraib A. Lecturer at Abbes Laghrour University. A voucher specimen has been deposited to the Herbarium of Laboratory of Natural Resources Valorization, Setif 1 University.

The essential oil of *J. thurifera* berries (100 g) was extracted by hydrodistillation with a Clevenger-type apparatus for 3 h.<sup>10</sup> The yield of EO was averaged over three experiments and calculated according to the dry weight of the plant material. The extracted oil was dried over anhydrous sodium sulfate and stored in sealed glass vials at 4-5°C prior to analysis.

### Chemical analysis of the essential oil

Chemical analysis was carried out using a GC apparatus (Thermo) equipped with a Bruker BR5-MS column (5 % phenyl methyl siloxane, 30 m long and 0.32 mm i.d., with 0.25 µm film thickness), coupled to a mass spectrometer (MS) type DSQII (Thermo) with a detector impact of electrons 70 eV. The carrier gas was helium at a rate of 1.2 mL.min<sup>-1</sup>; the injection volume was 0.1 µL; injector split mode 1:100. The initial temperature of the column was kept at 70 °C for 1 min, and programmed up to 300 °C at a rate of 10 °C.min<sup>-1</sup> and then kept constant at 300 °C (column cleaning step) for 5 min. The mass spectra of each compound were recorded across an m/z range of 40 to 500 m/z.

Identification of compounds was achieved by comparison of their mass spectra and retention times with those of standards (NIST 2008 v2.0/Xcalibur data system) and those in the literature.<sup>23</sup> Retention indices (RI) were calculated by estimating the retention times of the eluting peaks with those of a mixture of n-alkanes.

### Antibacterial activity

#### Antibiotics and strains

The three antibiotics, GEN: gentamicin (10µg/disc), AMX: amoxicillin (25µg/disc) and CFZ: cefazolin (30µg/disc), and the four bacterial strains, two Gram positive bacteria: [*Staphylococcus aureus* (*S. aureus*) ATCC 25923 and *Bacillus subtilis* (*B. subtilis*) ATCC 21332], and two Gram negative bacteria: [*Escherichia coli* (*E. coli*) ATCC 25922, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853], used in this experiment were obtained from Bacteriology Laboratory of Hakim Saâdan Hospital, Biskra-Algeria.

### Disc diffusion assay

The antibacterial activity of the *J. thurifera* EO alone (using several concentrations: 1/1, 1/2, 1/4, 1/8 and 1/16 in methanol) and in combination with antibiotics was assessed by disc diffusion method.<sup>24</sup>

The isolated colonies were picked from overnight grown cultures (18-24 h) in nutrient agar, inoculated in sterile saline solution by adjusting the turbidity to match 0.5 McFarland standards. Petri dishes (90 mm of diameter) were prepared with 20 mL of Mueller-Hinton Agar and seeded with 100 µL of the test bacteria (log phase cultures).

In the first experiment, sterile filter paper discs (6 mm in diameter) soaked with 10 µL of different EO concentrations were placed in the center of agar plate seeded with the respective bacteria. In the second experiment, the standard antibiotic discs (gentamicin, amoxicillin, cefazolin) soaked with 10 µL of selected concentrations of the essential oil were placed in the center of the respective plates of the test organisms. Plates were placed at 4 °C for 2 h and then incubated at 37 °C for 24 h. Standard discs of antibiotics (without EO) were used as positive control while discs soaked with methanol were used as negative control. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

The sensitivity to the different antibacterial solutions was classified by the diameter of the inhibition zone as: not sensitive for diameters less than 8 mm, sensitive for diameters of 9-14 mm, very sensitive for diameters of 15-19 mm and extremely sensitive, for diameters larger than 20 mm.<sup>25</sup>

### Statistical analysis

All experiments were carried out in triplicate. Data are expressed as mean ± SD. Differences were evaluated by one-way ANOVA test (0.5 %) using Statistica 8.0 software, StatSoft Inc., USA.<sup>26</sup> The combination of EO with ABs can provide synergistic, additive, or antagonistic interactions. If the value of combined EO/ABs is significantly higher ( $P < 0.05$ ) than the sum of individual values (after removing the disc diameters), it is considered to be a synergistic effect; and if it is equal ( $P \geq 0.05$ ), it is an additive effect. However, the antagonistic effect occurs when the value of one or both EO/ABs is significantly higher than the value of their mixture.<sup>27</sup>

## Results

### Chemical composition of the essential oil

Hydrodistillation of the ripe berries gave a faint green color essential oil with a mean yield of 1.38 % (v/w). Of the fifty components detected, thirty-five were successfully identified representing 86.93 % of the oil (Table 1). Six constituents were monoterpene hydrocarbons accounting for 31.89 % of the EO, fifteen constituents were oxygenated monoterpenes (26.5 %), four constituents were sesquiterpene hydrocarbons (3.4 %), and ten constituents were oxygenated sesquiterpenes (25.05 %). The major constituents identified in the oil

were m-mentha-6,8-diene (15.43 %),  $\beta$ -pinene (10.59 %), elemol (8.31 %), terpinene-4-ol (7.44 %),  $\alpha$ -cadinol (6.63 %) and linalool (4.64 %).

**Table 1.** Chemical composition of essential oil isolated from ripe berries of *Juniperus thurifera* var. *aurasiaca*.

RT	KI	Components	%
4.16	940	$\alpha$ -pinene	0.41
4.83	977	Sabinene	0.98
4.93	983	<b><math>\beta</math>-pinene</b>	<b>10.59</b>
5.12	994	$\beta$ -Myrcene	1.29
5.73	1028	O-Cymene	3.28
5.81	1033	<b>m-Mentha-6,8-diene</b>	<b>15.43</b>
6.48	1071	cis-Sabinene hydrate	1.96
7.04	1104	<b>Linalool</b>	<b>4.64</b>
7.4	1125	cis-p-Menth-2-en-1-ol	0.79
7.61	1138	cis-Limoneneoxide	0.58
7.72	1145	Camphor	0.74
8.01	1163	Sabina ketone	0.8
<b>8.33</b>	<b>1182</b>	<b>terpinene-4-ol</b>	<b>7.44</b>
8.45	1190	p-Cymen-8-ol	1.1
8.55	1196	$\alpha$ -terpeneol	1.15
9.35	1249	Carvone	0.79
<b>9.5</b>	<b>1259</b>	<b>Phellandral</b>	<b>2.37</b>
10.2	1305	Myrtenylacetate	0.42
10.8	1354	$\alpha$ -terpinylacetate	1.57
11.1	1369	2R,4R-p-Mentha-1(7), 8-diene-2-hydroperoxide	0.71
<b>11.3</b>	<b>1386</b>	<b>Nerolidylacetate</b>	<b>1.44</b>
11.5	1399	$\beta$ -Elemene	0.75
12.8	1505	$\alpha$ -Amorphene	0.55
13.1	1525	$\gamma$ -muurolène	1.24
13.5	1559	<b>Elemol</b>	<b>8.31</b>
13.6	1567	E-Nerolidol	0.48
13.9	1598	Caryophylleneoxide	1.37
14.5	1643	(-)-Spathulenol	0.74
14.5	1646	$\beta$ -Gurjunene	0.86
14.6	1654	t-Muurolol	1.84
14.7	1668	<b><math>\alpha</math>-cadinol</b>	<b>6.63</b>
15.4	1727	Junipercamphor	1.3
16.2	1801	8- $\alpha$ -Acetoxyelemol	1.19
16.8	1855	Isoaromadendreneepoxide	<b>2.44</b>
17.3	1908	$\alpha$ -Copaen-11-ol	0.75
<b>Total identified</b>			<b>86.93</b>
<b>Yield (g/100 g dry weight)</b>			<b>1.38</b>
<b>Monoterpene hydrocarbons</b>			<b>31.98</b>
<b>Oxygenated monoterpenes</b>			<b>26.5</b>
<b>Sesquiterpene hydrocarbons</b>			<b>3.4</b>
<b>Oxygenated sesquiterpenes</b>			<b>14.15</b>

RT, retention time; KI, Kovats index. The major constituents and the chemical groups of identified compounds with corresponding portion in analyzed essential oil are presented in bold.

### Antibacterial activity

As shown in Table 2, the oil tested alone showed notable antibacterial activity against *E. coli* and *S. aureus* at concentrations of 1/1, 1/2, 1/4 and 1/8 (v/v in methanol), with inhibition zone diameters ranging from 8 to 27 mm,

while *P. aeruginosa* and *B. subtilis* exhibited resistance to all tested EO concentrations.

The antibiotics sensitivity test revealed that *E. coli*, *S. aureus* and *B. subtilis* were sensitive to all the tested antibiotics, while *P. aeruginosa* was resistant to Amoxicillin and Cefazolin discs (Table 3). The same table also indicates that the combination of *J. thurifera* EO with antibiotics demonstrated synergistic, additive and antagonistic interactions, depending on the combination of EO and ABs. The combination of EO/GEN exhibited a synergistic effect against all bacterial strains tested as evidenced by the significant increase of the inhibition zone diameters. In contrast, the combination of berries EO with the other antibiotics displayed antagonistic interactions on *E. coli*, *S. aureus* and *P. aeruginosa*. However, the combined application of EO/AMX and EO/CFZ demonstrated synergistic and additive effects against *B. subtilis* respectively.

### Discussion

Plants have always been a source of inspiration for novel therapeutic drugs, as plant-derived medicines have made a significant contribution to human health.<sup>28,29</sup> The focus of this study was to characterize and evaluate the putative antibacterial and synergistic activities of the EO extracted from the berries of Algerian *Juniperus thurifera* var. *aurasiaca*.

Considering the yield and chemical composition of the obtained EO, our results differ from those reported by Hernandez et al<sup>14</sup> in that they found lower proportion of berries essential oil 0.13 % (v/w), with as main components limonene (84.32 %),  $\beta$ -myrcene (3.82 %),  $\alpha$ -pinene (3.48 %) and  $\alpha$ -terpinolene (1.39 %). They also differ from those of Zeraib et al,<sup>10,11</sup> who, by analyzing the essential oils extracted from the leaves of the same subspecies (var. *aurasiaca*) obtained respectively 0.40 % to 0.53 % (v/w) of yield with sabinene as the major component. However, our results were consistent with the fact that it is widely accepted that juniper berries contain a higher proportion of essential oils than leaves and wood.<sup>30,31</sup> Generally, the yield and composition of the plant essential oils are influenced by several factors such as the method of extraction, plant part used for sampling, plant age, genetic makeup and environmental condition.<sup>21</sup> Therefore, the disagreement of our results with those previously reported on *J. thurifera* berries,<sup>14</sup> or leaves<sup>10,11</sup> could be explained by any of these factors.<sup>10,18,21</sup>

**Table 2.** Antibacterial activity expressed as mean  $\pm$  SD of growth inhibition zone diameters (mm) of *J. thurifera* berries essential oil.

Strains tested	EO concentration (diluted in methanol)					
	Pure	1/2	1/4	1/8	1/16	ME
<i>E. coli</i>	24.33 $\pm$ 0.58	12.67 $\pm$ 0.58	<b>10.33<math>\pm</math>0.58</b>	8.0 $\pm$ 0.58	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
<i>S. aureus</i>	27.0 $\pm$ 1.0	24.67 $\pm$ 0.58	15.0 $\pm$ 1.0	<b>12.67<math>\pm</math>0.58</b>	7.0 $\pm$ 1.0	6.0 $\pm$ 0.0
<i>P. aeruginosa</i>	<b>7.33<math>\pm</math>0.58*</b>	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
<i>B. subtilis</i>	<b>6.0<math>\pm</math>0.0</b>	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0

\*The results illustrated in bold correspond to the concentrations selected for the test of the combination of *J. thurifera* berries EO with the ABs. ME, Methanol. When inhibition zone diameters = 6 mm, no inhibition.

**Table 3.** Antibacterial activity expressed as mean  $\pm$  SD of growth inhibition zone diameters (mm) of conventional antibiotics alone and in combination with *J. thurifera* berries essential oil.

Test substance	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>
GEN	28 $\pm$ 1.0	32 $\pm$ 1.0	19.67 $\pm$ 0.58	22.83 $\pm$ 0.29
AMX	26 $\pm$ 1.0	29.67 $\pm$ 0.58	6.0 $\pm$ 0.0	15.33 $\pm$ 0.58
CFZ	42 $\pm$ 1.0	40.67 $\pm$ 0.58	6.0 $\pm$ 0.0	28 $\pm$ 1.0
GEN + EO	34.67 $\pm$ 0.58	46 $\pm$ 1.0	24.33 $\pm$ 0.58	27 $\pm$ 1.0
AMX + EO	14.67 $\pm$ 0.58	28 $\pm$ 1.0	6.0 $\pm$ 0.0	20.33 $\pm$ 0.58
CFZ + EO	38.67 $\pm$ 0.58	32.67 $\pm$ 0.58	6.0 $\pm$ 0.0	28.33 $\pm$ 0.58
<b>P values</b>				
EO/GEN	0.007**	3.8 $\times$ 10 <sup>-4</sup> ***	0.0074 **	0.0022 **
EO/AMX	5 $\times$ 10 <sup>-6</sup> ***	2.3 $\times$ 10 <sup>-4</sup> ***	0.016*	4.4 $\times$ 10 <sup>-4</sup> ***
EO/CFZ	8.4 $\times$ 10 <sup>-5</sup> ***	3 $\times$ 10 <sup>-5</sup> ***	0.016*	0.643 ns
<b>Combination Effect</b>				
EO/GEN	Synergistic	Synergistic	Synergistic	Synergistic
EO/AMX	Antagonistic	Antagonistic	Antagonistic	Synergistic
EO/CFZ	Antagonistic	Antagonistic	Antagonistic	Additive

ns, no significant; \*, significant (P<0.05); \*\*, highly significant (P<0.01); \*\*\*, very highly significant (P<0.001). GEN, gentamicin; AMX, amoxicillin; CFZ, cefazolin; EO, essential oil.

Because of their complex bioactive composition, EOs interact with several targets at the same time, thus preventing pathogens from acquiring resistance.<sup>8</sup> Therefore, the interaction between EO and antibiotics can produce three types of effects, namely: synergistic, additive or antagonistic effects. Interestingly, the combined application of *J. thurifera* EO with gentamicin demonstrated synergistic effects against all tested bacteria. A synergistic effect can be produced if the constituents of a mixture affect different targets.<sup>36</sup> This could be explained by the fact that EO penetrates the microbial cell, presumably because of its hydrophobic character, alters the cellular functions,<sup>37</sup> increases membrane permeability,<sup>38</sup> which facilitates the penetration of gentamicin.<sup>39</sup> Once inside, gentamicin interrupts protein synthesis by blocking the 30S subunit of the bacterial ribosome. Also, EOs are often more effective against Gram-positive than Gram-negative bacteria due to the fact that the outer lipopolysaccharide layer of gram-negative bacteria limits the diffusion of hydrophobic compounds.<sup>32</sup> Our results are in agreement with these findings in that the combined addition of EO/GEN had greater effects on Gram-positive bacteria (*S. aureus*, *B. subtilis*). In contrast, the combination of products acting on the same target of the microorganism leads to antagonistic or additive effects.<sup>36</sup> Consistent with this, the antagonistic and additive interactions observed respectively between EO/amoxicillin, and EO/cefazolin could be explained by the fact that EO and these two antibiotics act on the same target of the bacterial cells (e.g., cell membrane). Nevertheless, the interpretation of such results requires some caution since growth media and culture conditions can influence the observed effects. In conclusion, this study provides, for the first time, important data on the chemical composition and biological activity of the essential oil of *J. thurefira* var. *aurasiaca* berries. The studied essential oil (alone or in combination with antibiotics) showed significant activity against almost all the tested bacterial strains. However, further studies should be carried out on its antibacterial activity using other bacterial groups and on its antifungal, antioxidant, cytotoxic and phytotoxic properties as well.

### Acknowledgements

This study was supported by the Ministry of Higher Education and Scientific Research of the Algerian People's Democratic Republic.

### Conflict of Interests

The authors claim that there is no conflict of interest.

### References

1. Sienkiewicz M, Łysakowska M, Kowalczyk E, Szymańska G, Kochan E, Krukowska J, et al. The ability of selected plant essential oils to enhance the action of recommended antibiotics against pathogenic wound bacteria. *Burns*. 2017;43(2):310-7. doi:10.1016/j.burns.2016.08.032
2. Silva LN, Zimmer KR, Macedo AJ, Trentin DS. Plant natural products targeting bacterial virulence factors. *Chem Rev*. 2016;116(16):9162-236. doi:10.1021/acs.chemrev.6b00184
3. Kwiatkowski P, Mnichowska-Polanowska M, Pruss A, Masiuk H, Dziecioł M, Giedrys-Kalemba S, et al. The effect of fennel essential oil in combination with antibiotics on *Staphylococcus aureus* strains isolated from carriers. *Burns*. 2017;43(7):1544-51. doi:10.1016/j.burns.2017.04.014
4. Pereira V, Dias C, Vasconcelos MC, Rosa E, Saavedra MJ. Antibacterial activity and synergistic effects between *Eucalyptus globulus* leaf residues (essential oils and extracts) and antibiotics against several isolates of respiratory tract infections (*Pseudomonas aeruginosa*). *Ind Crop Prod*. 2014;52:1-7. doi:10.1016/j.indcrop.2013.09.032
5. Moussaoui F, Alaoui T. Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants. *Asian Pac J Trop Biomed*. 2016;6(1):32-7. doi:10.1016/j.apjt.2015.09.024
6. Alaoui Jamali C, Kasrati A, Fadli M, Hassani L, Leach D, Abbad A. Synergistic effects of three Moroccan thyme essential oils with antibiotic cefixime. *Phytothérapie*. 2017. doi:10.1007/s10298-017-1107-2

7. Boonyanugomol W, Krairiwattana K, Rukseree K, Boonsam K, Narachai P. *In vitro* synergistic antibacterial activity of the essential oil from *Zingiber cassumunar* Roxb against extensively drug-resistant *Acinetobacter baumannii* strains. *J Infect Public Health*. 2017;10(5):586-92. doi:10.1016/j.jiph.2017.01.008
8. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. *Medicines*. 2017;4(3):58. doi:10.3390/medicines4030058
9. Yap PS, Yiap BC, Ping HC, Lim SH. Essential Oils, A New Horizon in Combating Bacterial Antibiotic Resistance. *Open Microbiol J*. 2014;8(1):6-14. doi:10.2174/1874285801408010006
10. Zeraib A, Ramdani M, Boudjedjou L, Chalard P, Figuredo G. Characterization and chemosystematics of Algerian thuriferous juniper (*Juniperus thurifera* L.). *J Appl Bot Food Qual*. 2014;87:249-55.
11. Zeraib A, Ramdani M, Boudjedjou L, Chalard P, Figuredo G. Chemical composition and antibacterial activity of *Juniperus thurifera* L. essential oils. *J BioSci Biotech*. 2014;3(2):147-54.
12. Gauquelin T, Bertaudière V, Cambededes C, Largier G. Le Jeneverier thurifere (*Juniperus thurifera* L.) dans les pyrenees: état de conservation et perspectives. *Acta Botanica Barcinonensia*. 2003;49:83-94.
13. Vela E, Schäfer PA. Typification de *Juniperus thuriferavar. africana* Maire, délimitation taxonomique et conséquences nomenclaturales sur le Genévrier thurifère d'Algérie. *Ecologia Mediterranea*. 2013;39(1):69-80.
14. Hernandez EG, Martinez CL, Villanova R. Determination by gas chromatography of terpenes in the berries of the species *Juniperus oxycedrus* L., *J. thurifera* L. and *J. Sabina* L. *J Chromatogr A*. 1987;396:416-20. doi:10.1016/s00219673(01)94085-3
15. Adams RP. Systematics of multi-seeded eastern hemisphere *Juniperus* based on leaf essentials and RAPD DNA fingerprinting. *Biochem Syst Ecol*. 1999;27(7):709-25. doi:10.1016/s0305-1978(99)00016-2
16. Adams RP, Mumba LE, James SA, Pandey RN, Gauquelin T, Badri W. Geographic variation in the leaf oils and DNA Fingerprints (RAPDs) of *Juniperus thurifera* L. from Morocco and Europe. *J Essent Oil Res*. 2003;15(3):148-54. doi:10.1080/10412905.2003.9712098
17. Barrero AF, Quilez del Moral JF, Herrador MM, Akssira M, Bennamara A, Akkad S, et al. Oxygenated diterpenes and other constituents from Moroccan *Juniperus phoenicea* and *Juniperus thurifera* var. *africana*. *Phytochemistry*. 2004; 65(17):2507-15. doi:10.1016/j.phytochem.2004.07.021
18. Achak N, Roman A, Alifriqi M, Adams PR. Effect of the leaf drying and sources on the essential oil composition of *Juniperus thurifera* L. var. *africana* (Maire.) from Tensift Alhouz Marrekch (Morocco). *J Essent Oil Res*. 2008;20(3):200-4. doi:10.1080/10412905.2008.9699990
19. Achak N, Roman A, Alifriqi M, Adams PR. Chemical studies of leaf essential oils of three species of *Juniperus* from Tensift Al Haouz-Marrakech Region (Morocco). *J Essent Oil Res*. 2009;21(4):337-41. doi:10.1080/10412905.2009.9700185
20. Mansouri N, Satrani B, Ghanmi M, El ghadraoui L, Aafi A, Farah A. Valorisation des huiles essentielles de *Juniperus thurifera* et de *Juniperus oxycedrus* du Maroc. *Phytothérapie*. 2010;8(3):166-70. doi:10.1007/s10298-010-0550-4
21. Bahri F, Harrak R, Achak N, Romane A. Chemical composition and antibacterial activities of the essential oils isolated from *Juniperus thurifera* L. var. *africana*. *Nat Prod Res*. 2013;27(19):1789-94. doi:10.1080/14786419.2012.755678
22. Satrani B, Ghanmi M, Mansouri N, Amusant N. Antioxidant properties of essential oils extracted from three species of moroccan junipers. *Environmental Science: An Indian Journal*. 2015;11(7):239-47.
23. Adams RP. Identification of essential oil components by Gas chromatography/Mass spectroscopy. USA: Allured Publishing Corporation; 2001.
24. Dias C, Aires A, Bennett RN, Rosa EAS, Saavedra MJ. First study on antimicrobial activity and synergy between isothiocyanates and antibiotics against selected Gram-negative and Gram-positive pathogenic bacteria from clinical and animal source. *Med Chem*. 2012;8(3):474-80. doi:10.2174/1573406411208030474
25. Ponce AG, Fritz R, del Valle C, Roura SI. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *LWT-Food Sci Technol*. 2003;36(7):679-84. doi:10.1016/s0023-6438(03)00088-4
26. Hill T, Lewicki P. Statistics: Methods Applications, Statsoft, Tulsa, OK. Electronic version is available at: [www.statsoft.com/textbook/k-nearest-neighbors/](http://www.statsoft.com/textbook/k-nearest-neighbors/).
27. Semeniuc CA, Pop CR, Rotar AM. Antibacterial activity and interactions of plantessential oil combinations against Gram-positive and Gram-negative bacteria. *J Food Drug Anal*. 2017;25(2):403-8. doi:10.1016/j.jfda.2016.06.002
28. Sacca-Sidi IYM, Alowanou GG, Olounladé PA, Nadège-Dedehou VFG, Hounzangbé-Adoté SM. *In vitro* combined effects of *Zanthoxylum zanthoxyloides* and *Newbouldia laevis* methanolic extracts on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. *Journal of Animal Health and Production*. 2016;4(4):128-33. doi:10.14737/journal.ljahp/2016/4.4.128.133
29. Roslindawani MN, Syafiqah AS, Jesse FFA, Effendy AW, Zamri-Saad M. Recombinant *Caseous lymphadenitis* vaccine with palm oil as adjuvant enhances the humoral and cell-mediated immune responses in rat model. *Journal of Animal Health and Production*. 2016;4(1):22-5. doi:10.14737/journal.ljahp/2016/4.1.22.25

30. Ennajjar M, Bouajil J, Lebrihi A, Mathieu F, Savagnac A, Abderraba M, et al. The influence of organ, season and drying method on chemical composition and antioxidant and antimicrobial activities of *Juniperus phoenicea* L. essential oils. *J Sci Food Agric*. 2010;90(3):462-70. doi:10.1002/jsfa.3840
31. Shanjani PS, Mirza M, Calagari M, Adams RP. Effects drying and harvest season on the essential oil composition from foliage and berries of *Juniperus excels*. *Ind Crop Prod*. 2010;32(2):83-7. doi:10.1016/j.indcrop.2010.03.003
32. Burt, S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol*. 2004;94(3):223-53. doi:10.1016/j.ijfodmicro.2004.03.022
33. Majouli K, Besbes Hlila M, Flamini G, Ben Jannet H, Kenani A. *In vitro* antibacterial activity of the *Hertia cheirifolia* L. essential oils. *Journal of Coastal Life Medicine*. 2016;4(11):865-7. doi:10.12980/jclm.4.2016j6-172
34. Shunying Z, Yang Y, Huaidong Y, Yue Y, Guolin Z. Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. *J Ethnopharmacol*. 2005;96(1-2):151-8. doi:10.1016/j.jep.2004.08.031
35. Rahman MM, Sultana T, Yousuf Ali M, Rahman MM, Al-Reza SM, Rahman A. Chemical composition and antibacterial activity of the essential oil and various extracts from *Cassiasophera* L. against *Bacillus* sp. from soil. *Arab J Chem*. 2017;10(2):S2132-7. doi:10.1016/j.arabjc.2013.07.045
36. Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. *Phytomedicine*. 2009;16(2-3):97-110. doi:10.1016/j.phymed.2008.12.018
37. Lopez-Romero JC, González-Ríos H, Borges A, Simões M. Antibacterial Effects and Mode of Action of Selected Essential Oils Components against *Escherichia coli* and *Staphylococcus aureus*. *Evid Based Complement Alternat Med*. 2015; 795435. doi:10.1155/2015/795435
38. Nazzaro F, Fratianni, F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*. 2013;6(12):1451-74. doi:10.3390/ph6121451
39. Rosato A, Piarulli M, Corbo F, Muraglia M, Carone A, Vitali ME, et al. *In Vitro* synergistic action of certain combinations of gentamicin and essential Oils. *Curr Med Chem*. 2010;17(28):3289-95. doi:10.2174/092986710792231996