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# CHEMICAL COMPOSITION OF THE ESSENTIAL OIL EXTRACTED FROM ALGERIAN POPULATIONS OF *JUNIPERUS SABINA* L.

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**Abstract:** Quantitative and qualitative analysis by GC-MS of the essential oil extracted from male and female foliages of *Juniperus sabina* from Algeria were been carried out. The yields of essential oils reached 0.86% for male and 0.65% for female. Fifty compounds were revealed and used in the oil characterization. The main compounds in oil of female foliage were sabinene, γ-terpinene, δ- 2-carene, and terpinene-4-ol. In addition, eight compounds were revealed in female plants only. On the other hand, sabinene and trans-sabinyl acetate were identified as the major constituents, and seven compounds were detected exclusively in the oil of male individuals. The oils of Algerian populations of *Juniperus sabina* revealed a chemical composition which is closer to the Iberians populations comparatively to those of central Europe and Asia. Two components i.e. safrole and methyl eugenol were detected in essential oil of Algerian and also reported by litterature data on Iberian populations.

**Keywords:** *Juniperus sabina*, chemical variability, GC-MS.

## INTRODUCTION

Morphological parameters have been for a long time the major source of information upon which plant taxonomy is based. The development of techniques in the field of chemical analysis has provided a new useful tool for plant identification where confusion exists regarding the use of morphological characteristics (Vasek and Scora, 1967; Tatro et al., 1973; Fretz et al., 1976).

By the end of the twentieth century and the beginning of the twenty-first, an important number of papers had dealt with the role of essential oil in the identification of plant taxa. The first works go back to the sixties: we can cite Von Rudloff (1963) on Canadian savin juniper; Banthrope et al. (1973) on the oil of the same plant in England; Akimov et al. (1976) on juniper from Mediterranean region. More recently, studies on essential oils of juniper have been very helpful in the revision of species and subspecies taxonomic positions (Adams et al., 1998; Adams et al., 2006).

The genus *Juniperus* comprises nearly 67 species which are almost originating from the northern hemisphere except *Juniperus procera* Hochst. ex Endl which grows in East Africa along the Rift mountains. *Juniperus* is divided into three sections: The *Caryocedrus* section represented by a single species; the *Oxcycedrus* section which comprises 11 species; and the *Sabina* section which comprises the remaining species (Adams, 2004; Adams, 2011; Farjon, 2001; Farjon, 2005).

In Algerian flora, the *Juniperus* species form a small group of five (05) taxa: *Juniperus oxycedrus* L. and *Juniperus communis* L. affiliated to the *Oxycedrus* section; *Juniperus sabina* L., *Juniperus phoenicea* L. and *Juniperus thurifera* L. affiliated to the *Sabina* section (Quezel and Santa, 1963; Maire, 1967).

Morphologically, *Sabina* section is subdivided into junipers with serrate leaf margins and junipers with smooth leaf margins. The first ones are roughly located in the western hemisphere. The shape of female cones (often called berries) and the number of seeds per berry are two morphological parameters used to describe eastern hemisphere junipers of the section *Sabina,* so two types of eastern hemisphere junipers are discriminated on the basis of such parameters: one seeded and ovoid berry with a noticeable pointed tip; multi-seeded and globose cone with irregular surface (Adams et al., 2006; Adams et al., 2007).

*Juniperus sabina* L. is a smooth leaf margined, multi-seeded cone species of the eastern hemisphere, it's a wild evergreen shrub belonging to the Cupressaceae family. It's widely distributed in Europe and Asia (Adams et al., 2006; Adams et al., 2007).

In Africa, its distribution is confined to small stands of the Djurdjura mountains, in Algeria. In such a restricted area, the number of individuals is limited to a few hundred. Such species is considered of least concern in the UICN list of protected species but its presence at its southernmost edge on the Djurdjura mountains might have evolutionary and ecological significance.

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Its geographical isolation from its other conspecifics might have led to its differentiation for morphological, biochemical and molecular characteristics including the chemical composition of essential oils, given that such marker proved to be useful in chemotypic discrimination of taxa, especially in coniferous groups.

Traditionally, plants of savin juniper have been used for cosmetic purposes and as spice in European cuisine. In many folk medicines, they have an abortive propriety (Pages et al., 1988), their oils, obtained by distillation of different parts of the plant (berries, leaves and wood), are applied for treatment of many diseases from leprosy, typhoid to tapeworm (Orhan et al., 2011).

To our knowledge, the volatile oils of Algerian savin juniper have not been analyzed so far. This study aims at:

1) Characterizing the essential oil of savin juniper from Algeria for the first time. With consideration of gender of individuals;

2) Providing a chemical profile of Algerian savin Juniper for comparisons with European and Asian populations of the species.

## MATERIAL AND METHODS

### Study area

*Juniperus sabina* is presently found on three small stands of the Djurdjura mountains: Issig-issig, Targa-ouroumi and Assoual.The substrate is calcareous in all stands and the elevation is around 1900 above sea level. The stands are completely open with savin juniper as the main shrub. In October 2016, twigs of 10 male individuals were collected from Targa-ouroumi and those of the same number of female ones collected from Issig-issig. The geographical coordinates of the sampling sites are given in Table 1.

**Table 1.**

The geographical coordinates of the sampling sites

| Sampling site | Latitude | Longitude | Altitude |
|---|---|---|---|
| Targa-ouroumi | 36°27'49'' N | 4°11'22'' E | 1850 m |
| Issig-issig | 36°28'35'' N | 4°05'53'' E | 2000 m |

The collected samples were sealed in plastic bags and brought in the same day into the laboratory where they were first identified using morphological characteristics, then washed and dried at ambient temperature for fifteen (15) days. A voucher specimen of the plant was deposited at the herbarium of the Laboratory of Botany, Faculty of Nature and Life Sciences, University of Bejaia, Algeria.

### Extraction of the essential oils

100g samples of male and female twigs were separately macerated in 400ml distilled water for 24hours and then subjected to a hydro-distillation for 4 hours using a Clevenger apparatus. The oil layer was separated from the aqueous one by decantation. Subsequently, the oil obtained was collected, dried over anhydrous sodium sulfate, transferred to sealed vials and conserved at -20°C until GC-MS analysis.

### The gas chromatography-mass spectrometry (GC-Ms) analysis of the essential oils

The GC–MS analysis of the essential oil of *Juniperus sabina* samples was performed using a GC–MS Shimadzu QP2010 equipped with a non-polar column SE30 (30 m . 0.22 i.d. mm. film thickness 0.25 µm). For GC–MS detection, a quadrupole detector type EI 70eV was used. Inert helium was used as a carrier gas at a constant flow rate of 0.77 ml. min$^{-}$1. Injector temperature was set at 250°C and 300°C. The oven temperature was programmed from 50°C to 180°C at 5 °C min$^{-}$1 and then held isothermally for 20 min and finally raised to 300°C at 10°C min$^{-}$1. 0.5µl samples were injected with a splitting ratio of 20.0 in split mode. Identification of the essential oil compounds was based on GC retention time, computer matching of mass spectra with standards (Nist 05 standard reference database of GC–MS systems).

## RESULTS AND DISCUSSIONS

### Essential oils yield

The essential oils isolated separately from twigs of male and female plants of *Juniperus sabina* were yellow and have a pungent odor. The female plants and the male ones yielded 0.65 %, and 0.86% (v/w dried material) of essential oil respectively. In Table 2 were indicated the yields of essential oils from different parts of *Juniperus sabina* harvested in various geographic regions. It showed that our results, as well as the results reported in the literature, varied in the same interval, they were situated between 0.6 % as the lowest limit and 2.8% as the highest content.

**Table 2.**

The yields of *Juniperus sabina* as reported in the literature

| Authors and country | Parts of plant | Yield (%v/w) |
|---|---|---|
| Netherlands: Koedam and Looman (1980) | TB | 2.8% |

| | | |
|---|---|---|
| China: Adams et al. (1998) | L | 1.5-1.9% |
| Iran: Emami et al. (2007; 2009) | LM<br>LF<br>F | 0.6%; 1.1%<br>1%; 0.75%<br>1.5%; 1% |
| Iran: Assili et al. (2010) | LM<br>LF<br>F | 0.6%<br>1%<br>1.5% |
| North of Iran: Asgary et al. (2013) | BMT<br>BFT<br>F | 1%<br>0.76%<br>0.92% |

Meaning of abbreviations: TB (terminal branches), L (leaves), LM (leaves male), LF (leaves female), F (fruits), BMT (branchlets of male tree), BFT (branchlets of female tree).

### Composition of the oil of female individuals

A GC-MS total ion chromatogram (TIC) of the essential oil obtained from female foliage of *Juniperus sabina* harvested at Issig Issig station is given in Figure1.

As can be seen in Table 3, about 80 peaks were revealed and fifty (50) compounds were identified representing about 86 % of the total chemical composition.

The constituents of the volatile oil are dominated by monoterpene hydrocarbons, followed by oxygenated monoterpenes and then by the fraction of sesquiterpenes. Sabinene was the main compound of the oil of of *J. sabina* female twigs with 16.69%. It is accompanied by γ-terpinene (11.60%), 2-carene (10.46%), terpinene-4-ol (9.93%), myrcene (5.92%), 3-carene (4.55%) and β-phellandrene (4.21%). Smaller amounts of α-pinene, α-phellandrene, methyl citronellate, D-limonene, α-thuyene, δ-cadinene, 6,7-dihydroeugenol, elemol and 1,4-cineol were also identified.

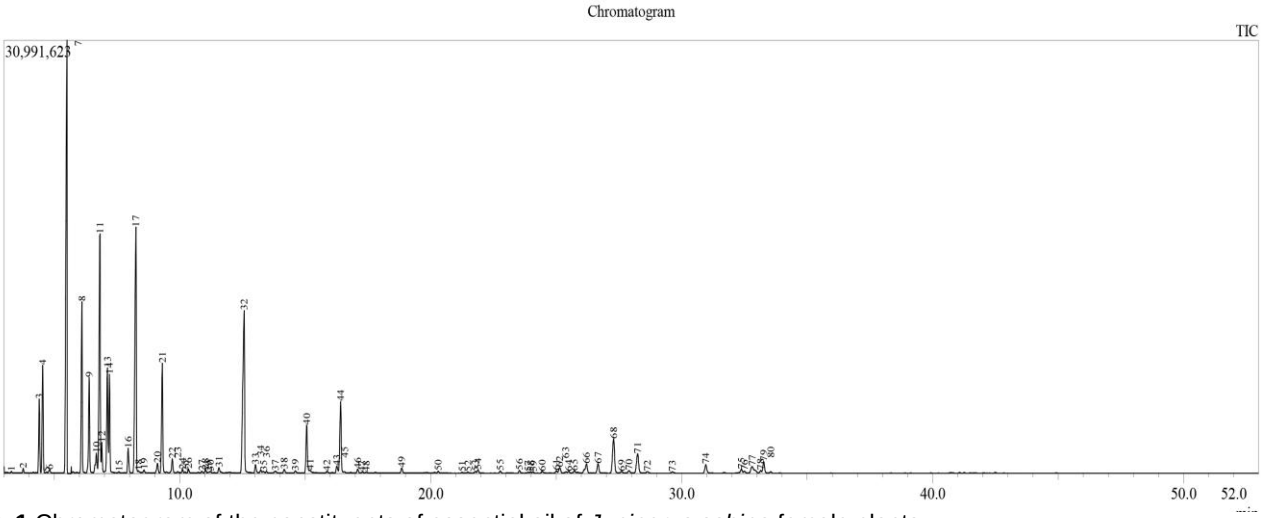


**Fig.1** Chromatogram of the constituents of essential oil of *Juniperus sabina* female plants.

Such compounds had moderate contents ranging between 1% and 4%. Twenty-five other compounds identified were present with weak concentrations varying between 0.1% and 1%. And finally, 6 components, having contents lower than 0.1%, were considered as traces.

It's although, important to emphasize the absence of trans-sabinyl acetate in the oil of Algerian females of *Juniperus sabina*.

**Table 3.**

Chemical composition of the essential oil of *Juniperus sabina* female plants

| N° | Compound | Peak number | Time retention | % |
|---|---|---|---|---|
| 1 | 2-ethyl-2-methyl-1,3-propanediol | 2 | 03.764 | 0.13 |
| 2 | α-thuyene | 3 | 04.400 | 2.59 |
| 3 | α-pinene | 4 | 04.538 | 3.85 |
| 4 | β-thujene | 5 | 04.693 | t |
| 5 | camphene | 6 | 04.814 | 0.15 |
| 6 | sabinene | 7 | 05.501 | 16.69 |

| | | | | |
|---|---|---|---|---|
| 7 | myrcene | 8 | 06.101 | 5.92 |
| 8 | α-phellandrene | 9 | 06.392 | 3.61 |
| 9 | 1,4-cineol | 10 | 06.680 | 1.09 |
| 10 | 2-carene | 11 | 06.820 | 10.46 |
| 11 | ρ-cymene | 12 | 06.893 | t |
| 12 | β-phellandrene | 13 | 07.117 | 4.21 |
| 13 | D-limonene | 14 | 07.201 | 3.27 |
| 14 | β-ocimene | 15 | 07.574 | t |
| 15 | cis-β-ocimene | 16 | 07.944 | 0.87 |
| 16 | γ-terpinene | 17 | 08.251 | 11.6 |
| 17 | cis sabinene hydrate | 18 | 08.360 | 0.03 |
| 18 | isolimonene | 19 | 08.574 | 0.13 |
| 19 | 3-carene | 21 | 09.305 | 4.55 |
| 20 | linalool | 22 | 09.703 | 0.71 |
| 21 | thujone | 23 | 09.920 | t |
| 22 | trans-rose oxide | 24 | 10.110 | 0.17 |
| 23 | trans sabinene hydrate | 26 | 10.347 | 0.16 |
| 24 | 1-terpineol | 27 | 10.880 | 0.09 |
| 25 | citronellal | 31 | 11.561 | 0.29 |
| 26 | terpinene-4-ol | 32 | 12.568 | 9.93 |
| 27 | α terpineol | 33 | 13.019 | 0.38 |
| 28 | cis-4-decenol | 34 | 13.227 | 0.12 |
| 29 | isopropenylanisole | 38 | 14.163 | 0.16 |
| 30 | α-citronellol | 39 | 14.607 | 0.11 |
| 31 | 6,7dihydroeugenol | 40 | 15.058 | 2.34 |
| 32 | piperitone | 41 | 15.200 | 0.12 |
| 33 | methyl citronellate | 44 | 16.410 | 3.35 |
| 34 | bornyl acetate | 46 | 17.092 | 0.16 |
| 35 | copaene | 51 | 21.253 | t |
| 36 | β-bourbonene | 52 | 21.483 | t |
| 37 | β-elemene | 54 | 21.885 | 0.16 |
| 38 | caryophyllene | 55 | 22.770 | t |
| 39 | germacrene B | 56 | 23.537 | 0.14 |
| 40 | β-cubenene | 60 | 24.435 | 0.12 |
| 41 | β-amorphene | 61 | 25.007 | 0.11 |
| 42 | γ-muurolene | 62 | 25.139 | 0.36 |
| 43 | β-cadinene | 65 | 25.721 | 0.10 |
| 44 | α -muurolene | 66 | 26.205 | 0.72 |
| 45 | δ-cadinene | 68 | 27.289 | 2.37 |
| 46 | elemol | 72 | 28.635 | 1.44 |
| 47 | β-eudesmol | 75 | 32.379 | 0.19 |
| 48 | tau-cadinol | 76 | 32.502 | 0.09 |
| 49 | α-eudesmol | 78 | 33.148 | 0.14 |
| 50 | α-cadinol | 79 | 33.269 | 0.68 |

Content values less than 0.1 are denoted traces (t)

### Composition of the oil of *Juniperus sabina* male individuals

In the second part of this study, a GC-MS total ion chromatogram (TIC) of the essential oil obtained from male plants of *Juniperus sabina* collected at Targa-ouroumi stand is presented in Figure 2. Nearly eighty (80) peaks were revealed and fifty (50) compounds were identified which represent about 90% of the total chemical composition. Three groups of constituents were determined. The first group was represented by monoterpene hydrocarbons; the second group was constituted by oxygenated monoterpenes and the third group contained the sesquiterpenic fraction. In previous study on Bulgarian savin juniper, Semerdjieva et al. (2019) found similar fractions.

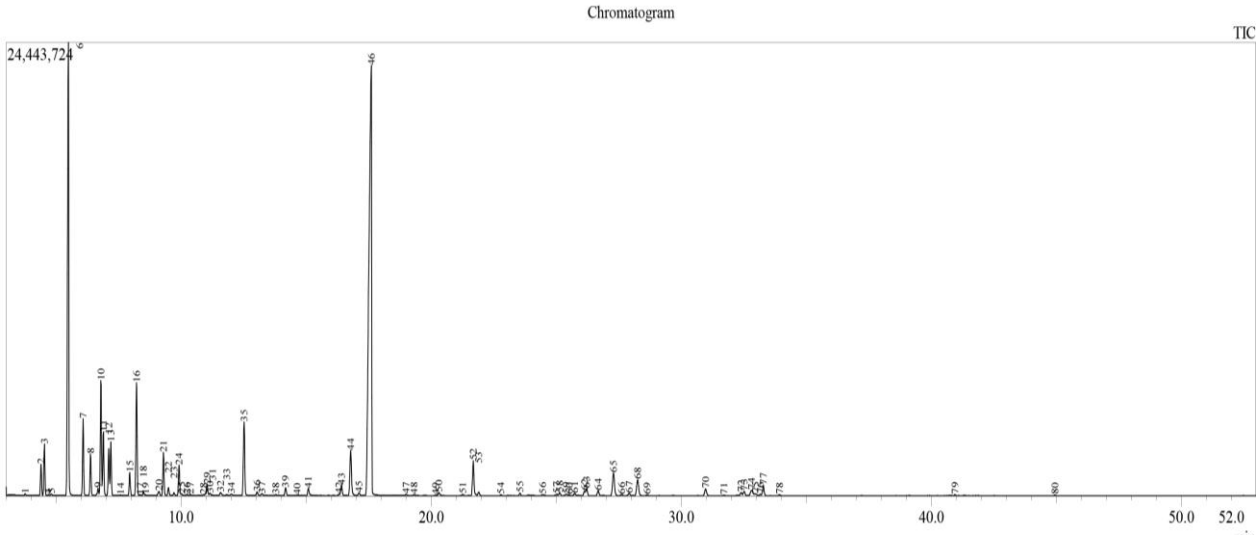


**Fig. 2:** Chromatogram of the constituents of essential oil of *Juniperus sabina* male plants.

As shown in Table 4, sabinene and trans-sabinyl acetate were the main compounds with the highest contents in the composition of the oil analyzed (18.40% and 37.79% respectively).

In adition to these two compounds, we identified small contents of other molecules which are in a deceasing order: 2-carene (4.45%), γ-terpinene (4.44%), terpinene- 4-ol (3.19), myrcene (2.68%), safrole (2.40%), ρ-cymene (2.32%), D-limonene (1.83%), β-phellandrene (1.81%), α-Pinene (1.73%), 3-carene (1.73%), methyl eugenol (1.71%), δ-cadinene (1.48%), thujone (1.26%), α-thujene (1.08%).

A third category of metabolites with contents fluctuating from 0.1% to 1% were identified, and consisted of: cis-β-ocimene (0.97%), α-cadinol (0.53%), methyl citronellate (0.50%), tau-cadinol (0.43%), 6,7 dihydroeugenol (0.39%), α.-muurolene (0.38%), p-isopropenylanisole (0.36%), 1,4-cineol, trans linalool oxide (0.32%), β-elemene (0.17%), α-terpineol (0.16%), γ-muurolene (0.15%), β-eudesmol (0.15%), bornyl acetate (0.13%), linalool (0.12%) and γ-elemene (0.11%). Finally, fifteen compounds were quantified and qualified as traces because of their very low rates less than 0.1%.

**Table 4.**

Chemical composition of the essential oil of *Juniperus sabina* male plants

| N° | Compound | Peak number | Time retention | % |
|---|---|---|---|---|
| 1 | 2-ethyl-2-methyl-1,3-propanediol | 1 | 03.761 | 0.06 |
| 2 | α-thujene | 2 | 04.394 | 1.08 |
| 3 | α-pinene | 3 | 04.531 | 1.73 |
| 4 | β-thujene | 4 | 04.688 | t |
| 5 | camphene | 5 | 04.810 | t |
| 6 | sabinene | 6 | 05.489 | 18.40 |
| 7 | myrcene | 7 | 06.079 | 2.68 |
| 8 | α-phellandrene | 8 | 06.376 | 1.53 |
| 9 | 1,4-cineol | 9 | 06.680 | 0.32 |
| 10 | 2-carene | 10 | 06.792 | 4.45 |
| 11 | ρ-cymene | 11 | 06.888 | 2.32 |
| 12 | β-phellandrene | 12 | 07.103 | 1.81 |
| 13 | D-limonene | 13 | 07.188 | 1.83 |
| 14 | tans-β-ocimene | 14 | 07.574 | t |
| 15 | cis-β-ocimene | 15 | 07.944 | 0.97 |
| 16 | γ-terpinene | 16 | 08.218 | 4.44 |
| 17 | cis sabinene hydrate | 17 | 08.360 | t |
| 18 | isolimonene | 19 | 08.573 | t |
| 19 | terpinolene | 20 | 09.110 | 0.22 |
| 20 | 3-carene | 21 | 09.293 | 1.73 |
| 21 | trans linalool oxide | 22 | 09.491 | 0.32 |
| 22 | linalool | 23 | 09.712 | 0.12 |
| 23 | thujone | 24 | 09.915 | 1.26 |
| 24 | carveol | 26 | 10.233 | t |
| 25 | trans sabinene hydrate | 27 | 10.366 | t |
| 26 | terpinene- 4-ol | 35 | 12.517 | 3.19 |
| 27 | α-terpineol | 36 | 13.037 | 0.16 |

| | | | | |
|---|---|---|---|---|
| 28 | cis-4-decenol | 37 | 13.235 | t |
| 29 | isopropenylanisole | 39 | 14.177 | 0.36 |
| 30 | α-citronellol | 40 | 14.640 | t |
| 31 | 6,7 dihydroeugenol | 41 | 15.084 | 0.39 |
| 32 | methyl citronellate | 43 | 16.412 | 0.50 |
| 33 | safrole | 44 | 16.781 | 2.4 |
| 34 | bornyl acetate | 45 | 17.111 | 0.13 |
| 35 | trans-sabinyl acetate | 46 | 17.607 | 37.79 |
| 36 | methyl eugenol | 52 | 21.681 | 1.71 |
| 37 | β-elemene | 53 | 21.901 | 0.17 |
| 38 | caryophyllene | 54 | 22.789 | 0.11 |
| 39 | γ-elemene | 55 | 23.549 | 0.11 |
| 40 | β-cubenene | 56 | 24.453 | t |
| 41 | β-amorphene | 57 | 25.019 | t |
| 42 | γ- muurolene | 58 | 25.140 | 0.15 |
| 43 | β-cadinene | 61 | 25.736 | 0.09 |
| 44 | α- muurolene | 63 | 26,215 | 0,38 |
| 45 | δ-cadinene | 65 | 27.290 | 1.48 |
| 46 | cadina-1,4-diene | 66 | 27.615 | t |
| 47 | β-eudesmol | 72 | 32.399 | 0.15 |
| 48 | tau-cadinol | 74 | 32.813 | 0.43 |
| 49 | α-eudesmol | 75 | 32.973 | 0.13 |
| 50 | α-cadinol | 77 | 33.282 | 0.53 |
| | | | | |

Content values less than 0.1 are denoted traces (t)

Quantities of oils presently obtained by hydro distillation of male and female plants in this study are comparable with values found by Asili et al. (2010); Nikolić et al. (2016); Zheljazkova et al. (2018).

The present study showed similar contents of sabinene in oils of both male and female plants (18.40% and 16.69 respectively).

Such values are concordant with those reported in many other studies (Von rudloff, 1963; Fretz et al., 1976; Koedam et al., 1980; Fournier et al., 1991; Adams et al., 2006; Emami et al., 2009; Nikolić et al., 2016). On the opposite, sabinyl acetate, the other main constituent of the oil extracted from male plants, was completely lacking in female plants. This is the most obvious difference between females and males which may be therefore chemically discriminated.

Many researchers reported the irregular occurrence of sabinyl acetate. For example, Fretz et al. (1976) did not find such compound in the oil of savin juniper collected at Secrest arboretum, Ohio; Adams et al. (2006) did not detect this molecule in the oil of Pyrenean population of savin juniper, but these sources did not specify the gender of individuals. While the absence of such a compound among female cones of savin juniper was reported by Emami et al. (2009) on Iranian populations; by Nikolić et al. (2016) on Macedonian ones and by Zheljazkova et al. (2018) on Bulgarian ones. Other differences concerning constituents with lower levels were observed in the present study. On one side, eight compounds (trans-rose oxide, 1-terpinol, citronellal, piperitone, copaene, β-bourbonene, germacrene B, elemol) were detected in oil extracted from females but were absent in males. On the other side, seven substances (terpinolene, trans-linalool oxide, carveol, safrole, methyl eugenol, γ-elemene, humulene, cadina-1,4-diene) were found to be specifically present in the composition of the oil from males. The noticeable disparities between females and males in the quantities of each constituent, especially the amounts of γ-Terpinene and 2-carene, might contribute to discrimination between females and males of savin juniper. In general, several factors have an important influence on the chemical composition of essential oils. These factors are genetic in relation to the variability inside the taxon as well as environmental, seasonal and geographical (Adams, 1970; Fretz et al., 1976).

## CONCLUSION

By the achievement of this analysis, we can establish that within Algerian populations of *Juniperus sabina* differentiation could be made between male and female individuals on the basis of their oils chemical composition. Female individuals were exempt from trans-sabinyl acetate, they had eight specific components and they had much more contents of γ-terpinene, terpinene-4-ol, 2-carene, myrcene and 3-carene. Meanwhile, male plants accumulated important concentrations of trans-sabinyl acetate and they can be recognized by the content of seven specific compounds. As expected, Algerian populations had a chemical profile closer to the Iberian population by the presence of safrole and methyl eugenol, those constituents have been revealed in the essential oils of Seirra Nevada an Pyrenean savin juniper, and they are lacking in central European and Asian plants.

It should be indicated that some compounds might be polymorphic within populations; consequently, they should be identified and the variations in their contents should not be taken as discriminating factors between different populations.

## AUTHORS CONTRIBUTIONS

Baya Farhi: laboratory manipulation, methodology, writing of original draft; Farid Bekdouche: supervision; Farid Bekdouche and Said Bouadam: field sampling;

Said Bouadam: identification of compounds and data processing; Fazia Krouchi: data validation, writing and editing; Arezki Derridj: proposition of the species and the problematic.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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