ABSTRACT
The species Senecio giganteus is endemic to North Africa, especially in streams of mound. The essential oil isolated from aerial parts of S. giganteus from Ain Roua (Setif) region was submitted to the hydrodistillation; the yield obtained is very low 0.02% (v/w). The oil was analysed by GC and GC/MS. The chemical analysis has allowed identifying 40 compounds corresponding to 92.38%. The oxygenated sesquiterpenes are dominant in the essential oil of S. giganteus with 21.22% and the hexadecanoic acid is the major component (17.80%), followed by isophytol (12.43%), pentanol 3 methyl (7.28%) and phytol (6.66%). The Essential oil of S. giganteus was tested for antimicrobial activity; it showed a modest effect against the strains tested. The karyological investigation of the population of S. giganteus has revealed a tetraploid chromosome number 2n = 4x = (20 + 2B), this number is reported for the first time in Algeria.

Keywords: Senecio giganteus, Essential oil, antibacterial activity, Chromosome, Algeria

INTRODUCTION
Senecionae is the largest tribe of the Asteraceae family gathered about 150 genera and 3000 species, nearly a third of the species of the tribe are included in the genus Senecio1-2. In Algeria, the Senecio genus is represented by 18 species of which five species are endemic3. Several species of the tribe are used in folk medicine4. The species of genus Senecio are known to be toxic but also for their beneficial effects on cough, eczema, bronchitis, wound healing and facilitation of childbirth5. S. vulgaris is used to calm painful menstruation and S. cineraria to relieve eye problems6. The essential oils of the genus have been many studies7-19. The butanol extracts of the flowers of S. giganteus present an antioxidant power20. The major components of oil in genus Senecio are highly variable; 1,10-epoxy furano-eremophilane (55.30%) found in the essential oil of S. aegyptius in Egypt; the curcumene (42.8%) in the oil of S. nemorensis of Turkey and the α-pinene (33.97%) in oil of S. graciliflorus from India21,22,15. The genus Senecio includes several chemical families; hydrocarbon compounds, aliphatic and the oxygenated compounds17. The chemical composition of essential oils from species of the genus Senecio is influenced by soil type and characteristics of the plant18. The essential oils of the genus Senecio have shown several biological activities. The oils of S. othonnae, S. nemorensis and S. racemosus, showed antimicrobial and antifungal activity20. The essential oil of S. aegyptius possesses an antifungal and antibacterial activity21. The essential oil of S. flavmeus is effective in the treatment of acute inflammations26; the essential oil of S. nudicaulis has an anti-oxidant power19, while the essential oil of S. rafineris presents an analgesic activity27. The essential oil of the flowers of S. graciliflorus has antioxidant potential and high cytotoxicity against cell lines of lung cancer19. The essential oil of S. graveolens has antibacterial and antifungal effects20, the oil of S. amplexicaulis exhibits antifungal activity23. The genus Senecio is largely polyploid with a basic number (x = 5)24-27. The species of the genus show a great chromosomal diversity28. The species with 2n = 10 are located in Africa and probably this is the site of original genus24,26,28. The highest chromosome number is observed in Senecio roberti-friesii with 2n = 36x = 180 chromosomes29. It is noteworthy that several species of the genus Senecio are wearing chromosomes B, (S. pogonias, S. sectilis, S. ragonesei, S. viridis, S. subalatus, S. uspallatensis, S. uspallatensis and S. uspallatensis)30. The aim of this work is to study the chemical composition, the antimicrobial activity and determination of chromosomal number of Senecio giganteus, a species endemic to eastern Algeria.

MATERIALS AND METHODS
Plant materials

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Senecio giganteus is an endemic plant of North Africa. Stems are fluted, with 2 cm in diameter. The leaves are whitish below; the flowers are yellow, grouped in corymbs. The akenes are very small, hairless (Figure 1). Senecio giganteus is collected from eastern Algeria, Ain roua (Setif) (Figure 2). Aerial parts were collected during the flowering stage in June 2014. The air dried materials were subjected to hydro-distillation for 3 h using a Clevenger apparatus type. Voucher specimens were deposited in the herbarium of the Department of Biology and Ecology, Setif University, Algeria. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in screw capped glass vials in a refrigerator at 4-5°C prior to analysis. Yield based on dried weight of the samples was calculated.

**Essential oil analysis**

The essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packard model 5971, equipped with a DB5 MS column (30 m X 0.25 mm; 0.25 μm), programming from 50°C (5 min) to 300°C at 5°C/min, with a 5 min hold. Helium was used as the carrier gas (1.0 mL/min); injection in split mode (1:30); injector and detector temperatures, 250 and 280°C, respectively. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; MS data were acquired in the scan mode in the m/z range 33-450. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library and those described by Adams, as well as on comparison of their retention indices either with those of authentic compounds or with literature values.

**Antimicrobial activity**

The antimicrobial activities of the essential oil of S. giganteus were evaluated against One Gram positive bacteria (Staphylococcus aureus ATCC25923), three Gram negative bacteria (Pseudomonas aeruginosa ATCC 27853, Klebsella pneumonia ATCC 70060, Escherichia coli ATCC 25922 and Shigella sp) and the yeast Candidat albicans ATCC 10231. The bacterial inoculums were prepared from overnight broth culture in physiological saline (0.8 % of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. Muller-Hinton agar (MH agar) and MH agar supplemented with 5 % sheep blood for fastidious bacteria were poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm Φ) were placed on inoculated agar, by test bacteria, filled with 10 μl of mother solution and diluted essential oil (1:1, 1:2, 1:4, and 1:8: v: v of DMSO). DMSO was used as negative control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. All tests were performed in triplicate. Then, Petri dishes were incubated at 37°C during 18 to 24h aerobically (bacteria). After incubation, inhibition zone diameters were measured and documented. The bactericidal and bacteriostatic tests on the five bacterial strains using pure oil of S. giganteus are performed in the present study.

**Caryology**

For karyotypic analysis, the squashing method is used. The root-tip meristems of from germinating seeds were usually used for chromosome preparations. A pre-treatment at room temperature for 1.5 hours was usually applied before fixation of the root-tips, in a 0.05% water solution of colchicine. After fixation in a cold mixture of ethanol acetic acid (3:1), the root-tips were stored in cold 70% ethanol until used. The following procedure involved the maceration in 45% acetic acid for 15 min, staining of chromosomes is made of emerging root-tips in acetic orcein with heating for one minute. Cutting off the meristems and squashing them in a drop of orcein.

**RESULTS**

The essential oil, of Senecio giganteus, isolated by hydro-distillation from the aerial parts, was obtained in very low yield 0.02% (v/w). The analysis by gas chromatography/mass spectrometry (GC-MS) (Figure 4 and 5) of the chemical composition of essential oils, we allowed the identification of 40 compounds in oil representing 92.38% of the total oil. The compounds, identified in this oil and their relative abundance, are presented in their order of appearance (Table 1). The major compounds of the essential oil of S. giganteus are hexadecanoic acid (17.80%), followed by isophytol (12.43%), 3-methyl pentanol (7.28%), phytol (6, 66%) and the spathululen (4.47%). Oxygenated sesquiterpenes are dominant in the essential oil of S. giganteus with 21.22%, followed by diterpenes (19.09%) and fatty acids with 17.80%. One Notices the poverty of monoterpenes compared to the sesquiterpenes and diterpenes. The antibacterial activity of the essential oil was determined by the disc diffusion method. Five strains bacteria and yeast and three controls (antibiotics) are used in this study (Table 2). The diameters of inhibition zones generated by the essential oil are well below those produced by antibiotics. The essential oil of S. giganteus shows moderate activity against Escherichia coli ATCC 25922 and Shigella sp. with a diameter of inhibition of (12-14mm). This oil is weakly active against Staphylococcus aureus ATCC 25923, Klebsella pneumonia ATCC 70060 and Pseudomonas aeruginosa ATCC 27853, with inhibition diameter of (7.5-11 mm). The yeast, Candidate albicans, is resistant to the essential oil of S. giganteus. The bactericidal and bacteriostatic tests on the five bacterial strains using the pure oil of S. giganteus show a bacteriostatic effect. To our knowledge S. giganteus, Algerian endemic species, has never been the subject of karyological study. The microscopic observation of the metaphase plates of root meristems, allowed to observe a tetraploid chromosome number, 2n = 4x = 20 + 2B, with the presence of two B chromosomes (Figure 5). This chromosome number is identified for the first time. The basic chromosome number of this species is x = 5.

**DISCUSSION**

The essential oil yield of S. giganteus in the region of Ain Roua (Setif) is considered very low (0.02%) compared with the performance of the population of Constantine (0.7%)42. While our result is inserted into the genus of
literature data, *S. aegyptius* from Egypt (0.05%)\(^7\), *S. polyanthemoides* of South Africa (0.07 %)\(^9\), *S. perralderianus* of Algeria (0.1%)\(^13\) and *S. graveolens* of Argentina (0.5%)\(^22\). The essential oil chemical profile of *S. giganteus* differs from other species of the genus. The chemical composition of *S. giganteus* of Constantine region (Algeria) shows similarity with our results, with the presence of five terpene compounds (eicosane, nerolidol<Z>, pentacosane, pentyl furan<2> and tridecene<1>)\(^14\). To our knowledge this is the only study that was done on the essential oil of this species. The chemical composition of *S. giganteus* is integrated to the overall context of the genus *Senecio*, by the presence of hexadecanoic acid, major compound in the genre, as in *S. flammeus*\(^16\). *S. giganteus* is characterized by the prevalence of oxygenated sesquiterpenes, moving closer to the chemical composition of species (*S. nudicaulis and *S. adenotrichius*)\(^35\), *S. rowleyanus* of Egypt\(^36\), *S. royelanus* of India\(^37\), *S. belgaumensis* of India\(^12\), *S. vulgaris* and *S. angulatus* of France\(^17\). While the chemical composition of the species *S. perralderianus*, *S. leucanthemifolius* and *S. atacamensis* is significantly different from the chemical composition of *S. giganteus*. The results of bacteriological analyzes of *S. giganteus* are generally similar to those in the literature. Essential oils of our species show moderate activity against *Escherichia coli* and *Staphylococcus aureus*. The same results are cited for species *S. graveolens* and *S. pedunculatus*\(^40,41\) and *S. othonnae* and *S. nemorensis*\(^10\). The essential oils of *S. pogonias* and *S. oreophyton* show antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae*\(^42\). While, the bacteria *Staphylococcus aureus*, *E. coli*, *K. pneumoniae* and *Pseudomonas aeruginosa* are resistant to the oil of *S. glaucus* of Egypt\(^43\). The yeast *Candida albicans* is resistant to the essential oil of *S. giganteus* by against it is sensitive to the essential oil of *S. pedunculatus*\(^46\). The oil of *S. glaucus* from Egypt shows moderate activity against *C. albicans*\(^43\). The karyological study of *S. giganteus* showed the presence of a tetraploid karyotype with \(2n = 4x = 20 + 2B\). (Magnification = HI 100X).
Table 1: Chemical composition of Senecio giganteus essential oil

<table>
<thead>
<tr>
<th>Chemical Class</th>
<th>Yield (%)</th>
<th>KI 0.02</th>
<th></th>
<th>Yield (%)</th>
<th>KI 0.02</th>
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<tbody>
<tr>
<td>Total</td>
<td>92.38</td>
<td></td>
<td>Total</td>
<td>92.38</td>
<td></td>
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<tr>
<td>Pentanol 3-methyl</td>
<td>833</td>
<td>7.28</td>
<td>Muurola-4(14),5-diene-trans</td>
<td>1493</td>
<td>1.17</td>
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<tr>
<td>Heptanal (2E)</td>
<td>947</td>
<td>3.40</td>
<td>Δ-amorphene</td>
<td>1511</td>
<td>0.65</td>
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<tr>
<td>Pentylfurran-2</td>
<td>984</td>
<td>0.64</td>
<td>Kessane</td>
<td>1529</td>
<td>0.54</td>
</tr>
<tr>
<td>Decene-1</td>
<td>986</td>
<td>1.42</td>
<td>Nerolidol (Z)</td>
<td>1531</td>
<td>2.19</td>
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<tr>
<td>β-oicimene (Z)</td>
<td>1032</td>
<td>0.45</td>
<td>Spathulenol</td>
<td>1577</td>
<td>4.47</td>
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<tr>
<td>β-oicimene (E)</td>
<td>1044</td>
<td>1.16</td>
<td>Caryophyllene oxide</td>
<td>1582</td>
<td>3.09</td>
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<tr>
<td>Octen-1-ol (3Z)</td>
<td>1047</td>
<td>0.52</td>
<td>Salvial 4(14) en 1 one</td>
<td>1594</td>
<td>2.06</td>
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<tr>
<td>n-octanol</td>
<td>1063</td>
<td>0.38</td>
<td>Humulene epoxide II</td>
<td>1608</td>
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<tr>
<td>Octenol (5Z)</td>
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<td>1.73</td>
<td>Himachalol</td>
<td>1652</td>
<td>0.67</td>
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<tr>
<td>Linalool</td>
<td>1095</td>
<td>1.09</td>
<td>α-cadinol</td>
<td>1652</td>
<td>0.03</td>
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<td>n-nonanal</td>
<td>1100</td>
<td>0.68</td>
<td>Amorpha-4,9-dien-2-ol</td>
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<td>α-terpineol</td>
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<td>Mint sulfide</td>
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<td>Dodecene-1</td>
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<td>Phytol</td>
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<td>Tetradecatriene (3Z,6Z,9Z)</td>
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<td>Isophytol</td>
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<td>Tridecene-1</td>
<td>1290</td>
<td>0.54</td>
<td>Hexadecanoic acid</td>
<td>1959</td>
<td>17.80</td>
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<tr>
<td>β-damascone (E)</td>
<td>1383</td>
<td>0.26</td>
<td>Eicosane (C20)</td>
<td>2000</td>
<td>0.87</td>
</tr>
<tr>
<td>Caryophyllene (Z)</td>
<td>1408</td>
<td>1.14</td>
<td>Tetracosane</td>
<td>2400</td>
<td>0.48</td>
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<tr>
<td>Neryl acetate</td>
<td>1434</td>
<td>1.03</td>
<td>Pentacosane</td>
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<td>2.65</td>
</tr>
<tr>
<td>α-humulene</td>
<td>1452</td>
<td>0.90</td>
<td>Hexacosane</td>
<td>2600</td>
<td>1.29</td>
</tr>
<tr>
<td>β-ionone (E)</td>
<td>1483</td>
<td>1.22</td>
<td>Nonacosane</td>
<td>2900</td>
<td>2.43</td>
</tr>
</tbody>
</table>

Table 2: Inhibition diameter of essential oil of Senecio giganteus

<table>
<thead>
<tr>
<th>Microbial strains</th>
<th>Controls</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Echerichia coli ATCC 25922</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Klebsiella pneumonia ATCC 70060</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Candida albicans ATCC 10231</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CN = gentamicine, CTX = cefotaxime, CS = colistin sulfate

controversial between x = 5 and (x = 10)²⁴-²⁵,²⁷, while x = 5 is confirmed for the genus Senecio²⁸.

CONCLUSION

The chemical analysis of the essential oil of Senecio giganteus show that the oil contains 40 compounds when the Hexadecanoic acid is the major constituent (17.80%), and reported that the oil is rich of oxygenated sesquiterpenes. This result is differing from the other species of genus Senecio. The testing of the antimicrobial activity of essential oils of S. giganteus shows that the oil has a moderate antibacterial activity and it was inactivated against yeast. The karyological study of S. giganteus based on chromosome counting, allows us to determine a tetraploid with 2n = 4x = 20 + 2B, with a basic chromosome number x = 5 and this result are reported for the first time in Algeria. This study thus reflects that Senecio giganteus could be considered as a potential natural source of oxygenated sesquiterpenes and the karyological results can help in the classification of the species and the genus Senecio.

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Competing interests
The authors declare that they have no competing interests

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