



Original Article

# Chemical Composition, Antibacterial Activity and Chromosome Number of *Helichrysum lacteum*, Endemic from Algeria

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The aim of this work is to investigate the chemical composition, antibacterial of essential oil and chromosome numbers from *Helichrysum lacteum* variety *typicum* endemic to Algeria. Samples of *H. lacteum* were collected in the flowering stage, in eastern Algeria locality. The air dried materials were subjected to a hydrodistillation which gave a viscous liquid with pale yellow oil. The chemical composition of the essential oil was analyzed by GC and GC / MS. A total 46 compounds representing 95.74% of the total oil were identified in *H. lacteum*. The chemical composition is dominated by the presence of major products, hexadecanoic acid (12.62%), caryophyllene (E) (10.58%), farnesyl acetone (5Z, 9E) (8.25%),  $\alpha$ -pinene (7.99%),  $\alpha$ -humulene (5.88%), nonanal-n (5.30%), humulene epoxide II (2.99%), caryolan-8-ol (2.82%),  $\alpha$ -cadinol-epi (2.67%),  $\gamma$ -dodecalactone (2.49%), octanal-n (2.14%). The Essential oil of *H. lacteum* was tested for five microorganisms. The oil showed modest effect against all the bacteria tested. The population of *H. lacteum* shows a tetraploïde chromosome number,  $2n = 4x = 28$ , with a basic number  $x = 7$ .

**Keywords:** *Helichrysum lacteum*, Endemic, Essential oil, Antibacterial activity, Chromosome number, Algeria.

## 1. INTRODUCTION

The genus *Helichrysum* derives its name from the Greek words *helios* (Sun) and *chrysos* (Gold) which is appropriate considering the attractive yellow flowers displayed by several species<sup>1</sup> commonly known as gold-everlasting or eternal flowers<sup>2</sup>. The genus belongs to the *Asteraceae* family and tribe *Inuleae*<sup>3</sup>. This large genus consists of approximately 500-600 species<sup>3,4</sup>. It is a cosmopolitan genus

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with 25 species native to the Mediterranean area<sup>5</sup>. In Algeria, the genus is represented by three species with *H. lacteum* variety *typicum* endemic to Algeria<sup>6</sup>. *Helichrysum lacteum* is an aromatic shrub of North Africa (Algeria, Morocco) with a white flowers; this species is represented in Algeria by an endemic variety *typicum*, it is found in forest clearings of cedars and mountain pastures and it is very rare<sup>6</sup>.

Species of the genus play an important role in traditional medicine, they are used to fight headaches; Inflammation, respiratory ailments, asthma, bronchitis, cough, infections and insomnia<sup>7-12</sup>. Also they have been used as an astringent, diuretic and expectorant and in the treatment of the liver, skin, nervous systems, and digestive disorders<sup>13</sup>. The Isolated phytoproducts of *Helichrysum* species are used in the food, cosmetic and pharmaceutical industries<sup>14</sup>.

Plants from the genus *Helichrysum* are producers of secondary metabolites<sup>7</sup>. The chemistry of the *Helichrysum* genus is complex, with a wide variety of chemical classes, among which are monoterpenes and sesquiterpenoids, diterpenoids and flavonoids<sup>20-21</sup>, triterpenoids and phenolic compounds<sup>22</sup> and flavonoides<sup>23-26</sup>.

Several studies have recently reported the chemical composition of *Helichrysum* essential oils of various origins, *H. italicum* from Algeria<sup>27-28</sup>, from Belgrade<sup>29</sup> and from Brazil<sup>30</sup>, *H. microphyllum* ssp *tyrrhenicum* and *H italicum* from Italia<sup>14,31-32</sup>, *H. italicum* and *H. stoechas* from Portugal<sup>33-34</sup>, *H. splendidum* from South Africa<sup>35</sup> and *H. oligocephalum* from Iran<sup>36-37</sup>.

Cytological studies of this genus have been restricted to a few studies of karyotypes and chromosome number, only 10-12% of the species have benefited from karyological studies<sup>38</sup>. The available chromosome data indicate that polyploidy played a significant role in the speciation and the evolution of the genus<sup>39-40</sup>. The chromosome number most commonly found in *Helichrysum* is  $2n = 28$ , mainly in the Mediterranean, Macaronesian and Asian species<sup>38</sup>. But some records of  $2n = 14$  for African species and  $2n = 56$  for some East Mediterranean and Asiatic species are also known<sup>41</sup>. The basic chromosome number in *Helichrysum* are  $x = 4$  and  $(x = 7)$ <sup>38</sup>. In the literature, the chromosome number  $2n = 42$  is published two times<sup>38,42</sup>.

To the best of our knowledge, the chemical composition of essential oil of *Helichrysum lacteum* has not been studied yet, as well as its antimicrobial properties and the chromosome numbers. The aim of this work was to investigate the chemical composition, antibacterial of essential oil and chromosome numbers from *Helichrysum lacteum* variety *typicum* growing in Algeria.

## 2. MATERIALS AND METHODS

### PLANT MATERIAL

Samples of *Helichrysum lacteum* L. were collected in the flowering stage, in eastern Algeria locality (Figure 1). Aerial parts were collected in October 2014.

*Helichrysum lacteum* variety *typicum* is a perennial, turf grass plant of 10-40 cm, characterized by globular Capitulos, nearly 1 cm long, pedunculate and gathered in dense corymb (Figure 2). The leaves are oblong or spatulate, tomentose-aranoseuse. The bracts of the involucre are silvery, milky, and brilliant, erect; Hairy at the base and matching flowers<sup>6</sup>. The air dried materials were subjected to hydrodistillation for 3h using a Clevenger apparatus type. Voucher specimens were deposited in the herbarium of the Department of Ecology and Biology, Setif 1 University, Algeria. The oil obtained was collected and dried over anhydrous sodium sulphate and stored at 4°C in sealed brown vials until use.

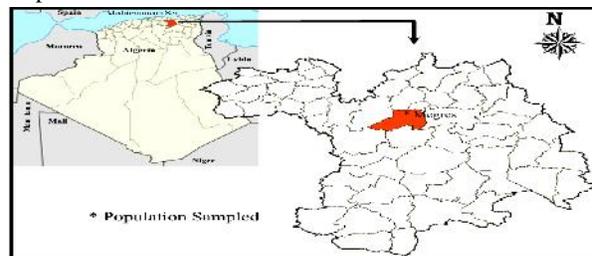


Fig 1: Population of *Helichrysum lacteum* sampled



Fig 2: *Helichrysum lacteum* of Megres region

### ESSENTIAL OIL ANALYSIS

The essential oils were analysed 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  $\mu$ m), 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<sup>43,44</sup> and those described by Adams, as well as on comparison of their retention indices either with those of authentic compounds or with literature values<sup>45</sup>.

### ANTIBACTERIAL ACTIVITY

One Gram positive bacteria (*Staphylococcus aureus* ATCC 2592) and four Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Shigella* sp) were

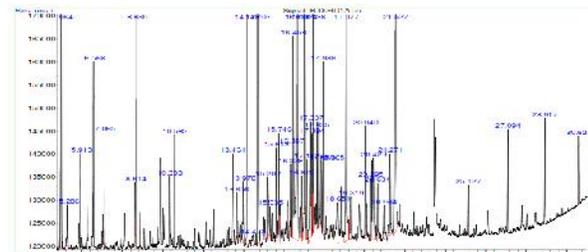
used in present study. The bacterial inoculums was prepared from overnight broth culture in physiological saline (0.8 % of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. Muller-Hinton agar (MH agar) was poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm) were placed on inoculated agar, by test bacteria, filled with 10 µl of mother solution and diluted essential oil (1:1; 1:2; 1:5 and 1:10 v/v of DMSO). DMSO was used as negative control. The antibiotic, gentamicin, cefotaxime and colistin sulphate was used as positive control. Then Petri dishes were incubated at 37°C during 18 to 24h aerobically. After incubation, inhibition zone diameters were measured and documented.

**KARYOLOGY**

The crushing process is used in the karyotype analysis. The meristems of the roots, resulting from the germination of seeds, are used for chromosomal preparations. A pre-treatment at room temperature for 1 hour 15 minutes was applied before fixation of the root-tips, in a solution of colchicine a 0.05%. After fixation in a mixture of ethanol and acetic acid (3: 1), the roots are stored in ethanol at 70° to the cold, until used. The staining procedure involves the maceration of roots in acetic acid 45% for 15 minutes. The staining of chromosomes is performed in emerging in the roots extremities in acetic orcein by heating for one minute; then cut the meristems and crush them in a drop of orcein.

**3. RESULTS**

The hydro-distillation of *Helichrysum lacteum* essential oil gave a viscous liquid with pale yellow oil. The average yield of essential oil of the sample is 0.03%. The analysis and identification of the components of the essential oil of this species was performed using the (GC/MS) (Figure 3 and 4). The compound identified in these oils and their relative abundances are presented in order of their appearance (Table 1). This analysis led to the identification of 46 components representing 95.74% of the total oil of *H. lacteum*.

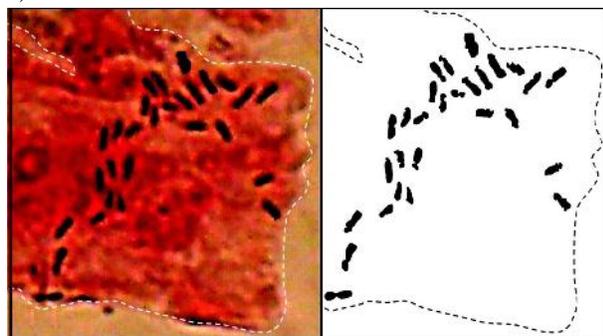


**Table 2: Inhibition diameter (mm) of *Helichrysum lacteum* essential oil**

| Microorganisms                          | Controls   |                  |            | Inhibition diameter* |      |      |     |
|---|------------|------------------|------------|----------------------|------|------|-----|
|   | Cefotaxime | Colistin sulfate | Gentamicin | Dilution             |      |      |     |
|   |            |                  |            | EO 1/2               | 1/5  | 1/10 |     |
| <i>Escherichia coli</i> ATCC 25922      | 33.5       | 15               | 25         | 10                   | 10.5 | 10   | 9.5 |
| <i>Staphylococcus aureus</i> ATCC 25923 | 18         | 18               | 0          | 10                   | 11   | 11   | 11  |
| <i>Klebsiella pneumoniae</i>            | 19         | 13               | 17         | 9                    | 10.5 | 9    | 9   |
| <i>Pseudomonas syringae</i>             | 18.5       | 15               | 22.5       | 9                    | 8    | 8    | 8   |
| <i>Shigella sp</i>                      | 14         | 15               | 30         | 13                   | 13   | 13   | 9.5 |

(\*): Average inhibition diameter (mm) of three trials

The observation of metaphase plates of *H. Lacteum* allowed us to identify a karyotype with a tetraploide chromosome number ( $2n = 4x = 28$ ) and a basic number ( $x = 7$ ) (Figure 5).



**Fig 5: karyotype of *Helichrysum lacteum* ( $2n = 4x = 28$ ). (Magnification = HI 100X).**

#### 4. DISCUSSION

In general, yields of essential oils of Algerian *Helichrysum* species are low. The yield of essential oil of the sample is 0.03%. It is similar with a yield of *H. italicum* subsp. *numidicum* 0.03%<sup>27</sup> and *H. italicum* subsp. *italicum* 0.02%<sup>30,46</sup>, 0.5 % for *H. italicum* subsp. *Microphyllum*<sup>47</sup>, 0.2% for *H. italicum*<sup>9,48</sup>, 0.44% for *H. italicum*<sup>28</sup>. The oil yield was 0.8% for *H. oligocephalum*<sup>36</sup>. These variations can be explained by the different climates, seasons, geographic and soil conditions, as well as differences in altitude and harvest periods of the plant.

The chemical profile of the essential oil of *H. lacteum* of Megres is different from other species reported by other authors in different regions. Our sample contains high percentages of hexadecanoic acid (12.62%), caryophyllene (E) (10.58%), farnesyl acetone (5Z, 9E) (8.25%), -pinene (7.99%), -humulene (5.88%), nonanal-n (5.30%), humulene epoxide II (2.99%), caryolan-8-ol (2.82%), -cadinol-epi (2.67%), -dodecalactone (2.49%), octanal-n (2.14%). The oil of *H. italicum* collected from the region of Bejaia (North of Algeria) contained mainly -cedrene, -curcumene, geranyl acetate, limonene, nerol, neryl acetate and -pinene<sup>28</sup>. The oils of *H italicum* collected in the regions of Bibans

(Algeria) have major compounds Isopropyl tetradecanoate, -pinene, hexadecanoic acid, caryophyllene (E), ledol, palustrol, -humulene, caryophyllene <9-epi>, -copaene, cis-calamenene<sup>27</sup>. On the other hand, In the Mediterranean region, several chemical compositions of essential oil of *Helichrysum* species have been observed<sup>14,29-30,34,36-37</sup>. The differences in chemical composition of essential oils between different species from genus *Helichrysum* are probably due to several factors, especially geographical factors, season and vegetation cycle<sup>49-50</sup>.

The bacteriological results of oil *H. lacteum* subsp. *typicum* from Algeria are similar to those of other Algerian species reporting in the literature. The methanolic and hexanic extracts of *H. stoechas* showed moderate antibacterial activity against *Bacillus cereus*, *Acinetobacter baumannii*, *Proteus mirabilis*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*<sup>51</sup>. The oil of *H. italicum* from Algeria inhibited the growth of all the tested microorganisms except three bacteria *Escherichia coli*<sup>28</sup>. Outer study showed that the oil of *H. italicum* has a modest effect against all the bacteria tested and its activity is important against the bacterium *S. aureus*<sup>27</sup>. *H. oligocephalum* oils from Iran showed significant antibacterial activity<sup>37</sup>. The extracts from *H. italicum* have the ability to inhibit the growth of *Staphylococcus sp*<sup>52</sup>.

The number of chromosomes most commonly observed in *Helichrysum* is  $2n = 28$  mainly in Mediterranean, Macaronesian and Asian species, with basic number  $x = 4$  and ( $x = 7$ )<sup>38</sup>. The number of chromosomes  $2n = 4x = 28$  with the basal number  $x = 7$ , is identified for the first time, for the species *H. lacteum*, subspecies *typicum*, endemic of Algeria. Our results agree with previous reports for the species of the genus, *H. italicum* subsp. *numidicum* from Algeria<sup>27</sup>. The same chromosome number is confirmed in *H. italicum* subsp. *silculum* from Italy<sup>41,38,53-55</sup>. The same result is obtained for *H. italicum* subsp. *Microphyllum* from Italy<sup>41</sup> and Spain<sup>55</sup>. This allows us to say that the chromosomal number of the *Helichrysum* genus at least for Algerian species is  $2n = 28$  (African species) with a basic number of  $x = 7$ .

#### 5. CONCLUSION

Analysis of the chemical composition of essential oils by GC/MS allowed the identification of 46 components in the essential oil of *Helichrysum lacteum* subsp. *typicum* collected from Megres region. It is characterized by the main presence of hexadecanoic acid (12.62%), caryophyllene (E) (10.58%), farnesyl acetone (5Z, 9E) (8.25%), -pinene (7.99%), -humulene (5.88%), nonanal-n (5.30%), humulene epoxide II (2.99%), caryolan-8-ol (2.82%), -cadinol-epi (2.67%), -dodecalactone (2.49%), octanal-n (2.14%). We also note that the chemical composition of the Megres sample differs from species of genus *Helichrysum* reported in the literature.

The antibacterial activity of *H. lacteum* subsp. *typicum* essential oils is tested on for bacterial strains. The results

show that the essential oil of this species has significant inhibitory action on almost all the bacteria tested. The chromosome number of *H. lacteum* subsp. *typicum* is stable and similar to bibliographic results. The chromosomal number of the Megres population is a tetraploid with  $2n = 4x = 28$ , and a chromosomal basic number  $x = 7$ .

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