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Research Article

Chemical Composition, Antibacterial Activity and Chromosome Number of *Helichrysum italicum* from Algeria

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ABSTRACT
The hydro-distillation of the essential oil of *Helichrysum italicum* variety numidicum *Pomel* gave a viscous liquid with pale yellow oil. The average yield of essential oil of the samples is 0.03%. The chemical composition of essential oil was analyzed by GC and GC/MS. A total 43 compounds representing 92.9% of the oil were identified in *H. italicum*. The chemical composition is dominated by the presence of major products, Isopropyl tetradecanoate (12.10%), α-pinene (12.02%), Hexadecanoic acid (9.96%), Caryophyllene (E) (9.22%), Ledol (9.11%), Palustrol (5.55%), α-Humulene (4.28%), Caryophyllene <9-epi> (3.75%), α-copaene (3.72%), cis-Calamenene (2.18%). The Essential oil of *H. italicum* was tested for six microorganisms. The oil showed modest effect against all the bacteria tested and it has no significant antifungal activity against the *Candida albicans.* The population of *Helichrysum italicum* shows a tertraploïde chromosome number of 2n = 28 with basic number x = 7.

Keywords: *Helichrysum italicum*, Essential oil, Antibacterial activity, Chromosome, Algeria.

INTRODUCTION

The genus *Helichrysum* (Miller) belongs to the *Asteraceae* family. The genus *Helichrysum* is distributed in the Mediterranean region[1]. The species *Helichrysum italicum* (Roth) G. Don is an aromatic shrub, endemic Mediterranean plant[2]. This species is represented in Algeria by an endemic variety (*numidicum* Pomel)[3] (Figure 1). *Helichrysum italicum* has a high economic interest due to its essential oil very sought out by the perfume industry and aromatherapy[2,4]. The Dried flowers of this species had a great reputation in traditional medicine as choleretic, diuretic, cholagogue, expectorant[5-7]. Many works report that the essential oil of *H. italicum* is an antihematome powerful[8], so its flowers and leaves are the most used parts in the treatment of health disorders such as allergies, colds, cough, toothache, liver and gallbladder disorders, respiratory and ENT, Digestive disorders, inflammation, infections and sleeplessness[9-19]. The genus is an important source of secondary metabolites[20]. The most analyzed extract of *H. italicum* is the essential oil[4]. There are many studies of the chemical composition essential oils of *H. italicum* grown in France[21-24], in Italy[22], in Portugal[28], in Croatia[29,30], in Belgrade[31], in Brazilia[32]. The chemical composition of essential oils of *H. italicum* in the world is highly variable (Table 1). The major components, having significant variability are α-pinene, neryl acetate, γ-curcumene, limonene, curcumene-ar, β-selinene and α-selinene. It should be noted that the chemical composition of essential oil of *H. italicum* shows a high level of intra-specific differences in response to plant genetics[33], environmental factors[34-36], geographical origin and the growing cycle[21,25]. The biological activities of the many metabolites isolated from *H. italicum*, and especially its volatile fraction have been found to display pharmacological properties, such as anti-inflammatory[8,20,37], antiallergic[8,38], antioxidant[39-42], antiviral activity and anti-HIV properties[20], antimicrobial activity[29,43-44] and antifungal properties[29,38]. Cytological studies of this genus have been restricted to a few studies of chromosome numbers and karyotypes, only 10-12% of the species are studied[45]. The available chromosome data indicate that polyploidy played a significant role in the speciation and the evolution of the genus[46-48]. The chromosome number most commonly found in *Helichrysum* is 2n = 28, mainly in the Mediterranean, Macaronesian and Asian species[45]. But some records of 2n = 14 for African species and 2n = 56 for some East Mediterranean and Asiatic species are also known[48]. The basic chromosome number in *Helichrysum* are x = 4 and x = 7[45]. In the literature, the chromosome number 2n = 42 is published two times[48-49]. The chromosome number of 2n = 28 has been identified in *H. italicum* Subsp. *Microphyllum* from Italy[48] and Spain[5].The same chromosome number is confirmed in *H. italicum* subsp *silculum from Italy*[48,50-52]. The aim of these studies is to identify the chemical composition of *H. italicum* (variety *numidicum* Pomel) essential oil and to compare the results to bibliographic data in the world as well as the identification of the

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Figure 1: *Helichrysum italicum* var. *numidicum* Pomel (Photo Bouchaala, 2015).

geographical distribution of chemotypes. Furthermore, the investigation of the antibacterial activity of the essential oil and the chromosome count of this species.

MATERIELS AND METHODES

Plant material

Samples of *Helichrysum italicum* were collected in the flowering stage, in eastern Algeria locality (Figure 2). Aerial parts were collected in October 2014. 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, Ferhat Abbas University, Algeria. The oil obtained was collected and dried over anhydrous sodium sulphate and stored at 4°C in sealed brown vials until use.

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 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[53,54], and those described by Adams, as well as on comparison of their retention indices either with those of authentic compounds or with literature values[55].

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* ATCC700603 and *Shigella* sp) and the yeast *Candida albicans*, 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 10µl 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.

Statistical analysis

Cluster analysis (UPGMA) was carried out on the original variables and on the Manhattan distance matrix to seek for hierarchical associations among the populations. The cluster analyses were carried out using STATISTICA 10 software.

RESULTS AND DISCUSSION

The hydro-distillation of the essential oil of *H. italicum* subsp. *numidicum* gave a viscous liquid with pale yellow oil. The yield of essential oil of the sample is 0.03%. It is similar with a yield of *H. italicum* subsp. *Italicum* 0.02%[26,32]. This yield is low compared to those of literature. The oil yield was 0.2% for *H. italicum*[2,30], 0.5 % for *H. italicum* subsp. *Microphyllum*[1] and they show that the yield of *Helichrysum* varies according to geographical location. The analysis and identification of the components of the essential oil of this species was performed using the (GC/MS). The compounds identified in the oil and their relative abundances are presented in order of their appearance (table 2). This analysis led to identification of 43 compounds representing 92.99 % of the total oil of *H. italicum*. The chemical composition of *H. italicum*'s essential oil in the world shows a high level of intraspecific differences, this is a response to environmental factors[34,35], especially soil properties[36], geographical origin, the growing cycle[21,25] and genetic[33]. This variability is so pronounced, that the UPGMA analysis did not brought out homogeneous groups, so that we can identify the different chemotypes composing this species. The classification of our populations, according to their chemical kinship relations, is based on the construction of clades. The UPGMA based on the Unweighted pair-group average

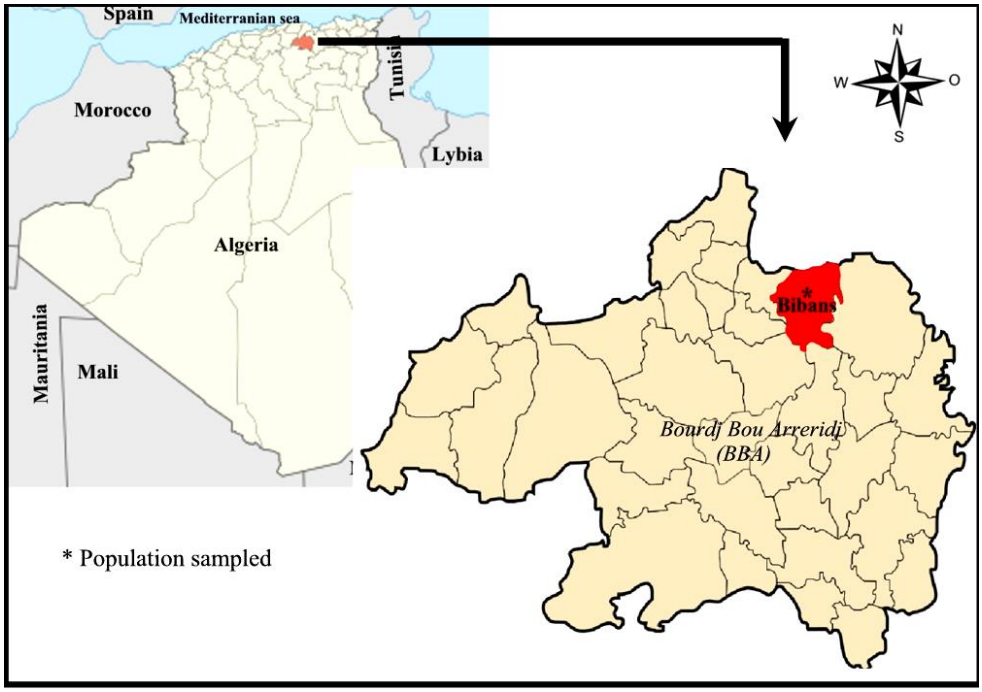

Figure 2: Population of *Helichrysum italicum* sampled

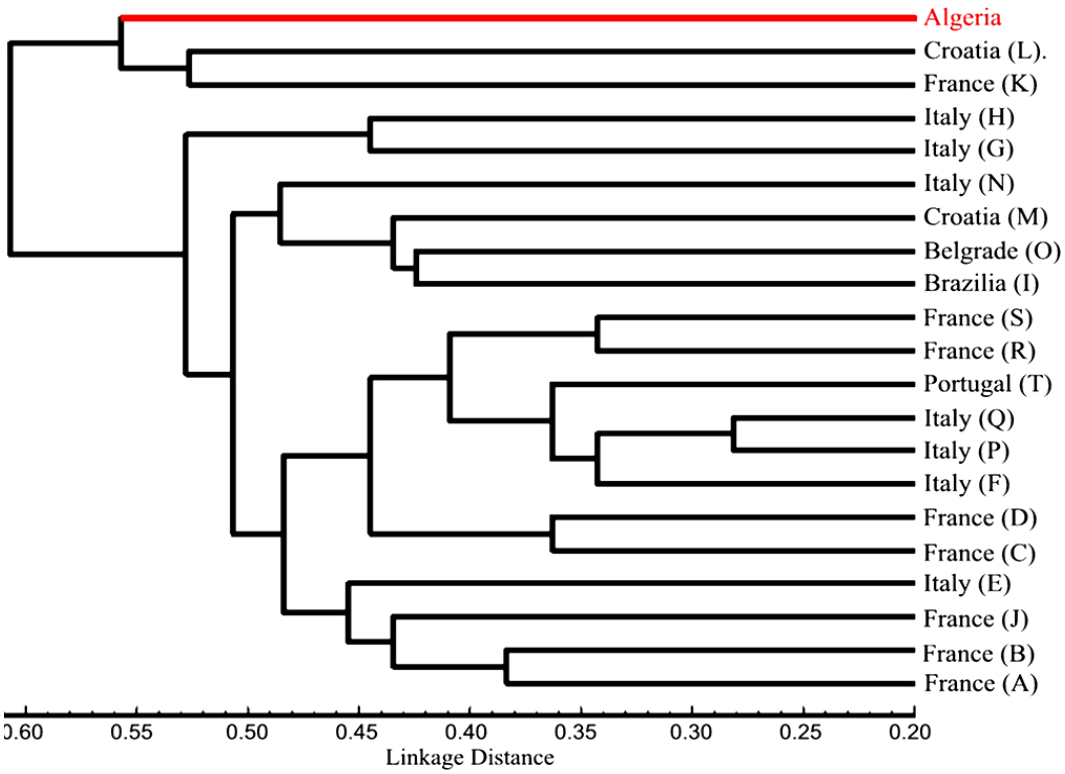

Figure 3: UPGMA cluster of *Helichrysum italicum* population.

distance and the City-block (Manhattan) (Figure 3), has divided the populations into two clades. Although this is the same species of *H. italicum*, it exhibits enormous difference in the chemical composition of essential oils. Our sample is separated from that of France and the Croatia in the first clade. We noted the individualization of *H. italicum* population studied. This population is rich in isopropyl tetradecanoate (12.10%), α-pinene (12.02%), hexadecanoic acid (9.96%), caryophyllene (E) (9.22%), Ledol (9.11%). It opposed to the populations of France[2] and the Croatia[29], which are characterized by 2-methylcyclohexyl pentanoate (11.1-8.3%) and neryl acetate (10.4- 11.5%). These populations are close of our population by the presence of α-pinene (12.8-11.2%). The antibacterial activity of the essential oils was evaluated using six microorganisms, using disc diffusion method. The diameters of inhibition zones of essential oils for the microorganisms tested are grouped in the Table 3. The

Table 1: Chemical composition of *Helichrysum italicum* in the word.

Subsp.	*Italicum*									*Helichrysum italicum (Roth) Guss. Don*						*microphyllum*				*picardii*
Country	France				Italy				Brazil	France		Croatia		Italy	Belgrade	Italy			France	Portugal
Authors	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1,4,6,9-Trimethyldec-8-en-3,5-dione	–	–	–	–	–	–	–	–		4.1	–	–	–	0.2	–	–	–	–	–	–
2,4,6,9-Tetramethyldec-8-en-3,5-	2.2	2.6	–	–	4.5	–	5.7	2.6	–	4.4	–	–	–	–	–	–	–	–	–	–
2-Methylcyclohexyl octanoate	–	–	–	–	–	–	–	–	–	–	3.4	4.8	–	–	–	–	–	–	–	–
2-Methylcyclohexyl pentanoate	–	–	–	–	–	–	–	–	–	–	11.1	8.3	–	–	–	–	–	–	–	–
Hexanedione <3,4>	–	–	–	11.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3,5,7,10-Tetramethylundec-9-en-4,6	–	–	3.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4,6,9-Trimethyldec-8-en-3,5-dione	3.7	5.6	11	6.2	4.6	–	14.7	8.8	–	–	–	–	–	–	–	–	–	–	–	–
4,6-Dimethyloctan-3,5-dione	1.3	2	2. 4	0.6	–	–	1	1.4	–	0.9	–	–	–	–	–	–	–	0.8	1.1	–
5,7,10-Trimethylundec-9-en-4,6-	1.7	2.6	6.9	2.6	0.4	–	0.6	0.3	–	–	–	–	–	–	–	–	–	–	–	–
Cedren-13-<8-ol>	–	–	–	–	–	4.2	–	–	–	–	6.8	–	–	–	–	–	–	–	–	–
Curcumene <ar->	2.3	1.8	6.4	6.4	3,5	–	1.9	1.5	8.3	1.5	2.3	–	4	0.1	1.9	1	0.1	3.9	2.6	2.8
Aromadendrene	–	–	–	–	–	–	–	–	–	–	–	4.4	–	–	–	–	–	–	–	–
Carvacrol	–	–	1.9	9.8	–	–	–	–	–	–	–	–	–	–	–	–	–	1.5	1.5	–
Bergamotene <α-cis->	–	–	–	–	0.5	–	0.7	0.3	–	0.6	–	–	2.2	–	1.4	–	–	1.6	–	0.2
Eremophilene	–	–	–	–	–	–	–	–	–	–	4.3	–	–	–	–	–	0.1	–	–	–
Eudesm-5-en-11-ol	0.8	3.5	–	–	2.3	–	1.8	1.7	–	3.4	–	–	–	2	–	–	–	–	–	–
Guaiol	0.5	1	–	–	1.1	2.3	0.6	0.3	–	–	2	0.6	–	0.3	–	–	0.2	1.1	3.1	0.2
Hexadecene	–	–	–	–	–	9.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Italicene <iso->	0.7	0.3	0.4	0.5	0.8	16. 8	0.2	0.2	3.2	0.4	–	–	–	–	–	–	1.2	–	0.3	0.3
Italicene	2.4	1.2	0.9	–	3	–	0.7	0.6	–	1.3	–	–	4.6	–	5.4	1.1	–	1.2	1.3	1.2

Table 1: Chemical composition of *Helichrysum italicum* in the word.

Subsp.	*Italicum*									*Helichrysum italicum (Roth) Guss. Don*						*microphyllum*				*picardii*
Country	France				Italy				Brazil	France		Croatia		Italy	Belgrade	Italy			France	Portugal
Authors	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
Limonene	6.9	2.9	–	–	4.6	–	1.9	7.6	–	4.5	4	3.8	0.6	4.5	2.5	3.5	0.8	–	–	0.5
Linalool	2.4	2.1	3.9	0.5	2.7	–	3.9	1.1	–	2.1	–	1.4	0.4	1.4	0.5	1.7	2.2	3.3	2.2	0.1
Manool	–	–	–	–	–	–	–	–	–	–	–	–	4.2	–	–	–	–	–	–	–
Neryl acetate	36.3	35	32	8.5	31	–	18.7	33.3	29.2	35.8	10.4	11.5	3.1	1.5	7.9	73.7	60.3	55.7	41.5	–
Neryl isovalerate	–	–	2.5	1.6	0.4	0.5	–	–	0.7	–	–	–	–	–	0.5	1.9	–	–	–	
Nerol	3.2	4.2	3.9	18.8	4	–	1.4	2.2	0.7	2.7	1.1	0.2	0.2	–	0.8	–	6.8	4.4	13.1	–
Neryl propanoate	4.8	4.9	3.6	6.8	5.1	–	4.7	3.8	10.1	3.4	0.7	–	–	0.4	1.4	3.7	5.4	12.7	5.6	–
*C*aryophyllene <trans>	–	–	–	–	–	–	–	–	2.8	–	5.4	1.1	6.9	7.9	4.7	–	–	–	3.8	–
Bergamotene <α-trans>	–	–	–	–	0.3	4.7	0.6	0.3	0.7	3.3	–	–	–	0.2	3.2	–	–	–	–	0.6
Amorphene <α->	–	–	–	3.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cedrene <α->	–	–	–	–	–	–	–	–	2.4	–	–	1	1	–	–	–	–	–	–	–
Copaene <α->	–	–	–	–	–	–	–	0.1	–	–	1.2	0.2	3.6	0.7	3.5	–	–	–	–	-
Eudesmol <α->	0.2	1	–	–	0.9	0.9	0.3	0.4	–	2.3	2.2	–	–	0.3	–	–	–	1.3	2.9	–
Pinene <α->	2.7	1.7	–	–	1.8	–	2.0	4.2	0.4	0.8	12.8	10.2	2.8	53.5	15.9	–	–	–	–	53.5
Selinene <α->	–	–	–	–	–	–	0.6	0.6	3.9	–	–	0.4	5.9	5.2	4.8	–	–	0.4	0.3	1.0
Terpineol <α->	0.7	1.7	1.0	2.3	0.5	–	1.3	2.1	1.1	0.9	2	1.4	0.49	0.8	0.26	–	–	0.9	1.5	0.4
Caryophyllene <β->	–	–	–	2.7	–	–	–	–	1.7	–	–	4.2	–	5.7	–	–	–	–	–	–
Costol <β->	–	–	–	–	–	7.5	–	–	–	–	–	-	–	–	–	–	–	–	–	–
Eudesmol <β->	0.4	0.8	–	–	1.3	1.4	–	–	–	2.5	3.5	0.5	1.1	–	–	0.7	0.2	1.9	–	–
Selinene <β->	–	–	–	–	–	–	0.5	0.8	1.6	–	2	1.6	9.9	–	6.9	–	–	0.5	0.4	–
Curcumene <γ->	3.7	–	5	–	10.7	–	11.1	8.6	18.8	6.6	1.1	–	12.4	1.4	22.5	3.7	12.9	2.5	8.2	27.4
Gurjunene <γ->	–	–	–	–	–	–	–	–	–	–	2.4	1.0	–	–	–	–	–	–	–	–
Cadinene <δ->	–	–	–	0.6	–	–	0.2	0.1	0.9	–	0.6	0.5	0.9	–	1.5	0.0	3.9	–	–	0.3

A, B-[21]; C, D-[23]; E-[24]; F-[26]; G, H-[25]; I-[32]; J-[22]; K-[2]; L-[29]; M-[30]; N-[22]; O-[31]; P, Q-[27]; R, S-[1]; T-[28]. Table data are used in the UPGMA analysis.

Table 2: Chemical composition of *Helichrysum Italicum* essential oil.

Total	KI	92.99	Total	KI	92.99
Number of compounds		43	Number of compounds		43
Yield (%)		0.03	Yield (%)		0.03
α-pinene	932	12.02	Palustrol	1567	5.55
β-pinene	974	0.67	Thujopsan-2-α-ol	1586	0.08
Limonene	1024	0.20	Caryophyllene oxide	1582	1.14
β-phellandrene	1025	0.80	Gleenol	1586	0.36
n-nonanal	1100	0.32	Ledol	1602	9.11
n-decanal	1201	0.63	Humulene epoxide II	1608	0.18
α-Copaene	1374	3.72	Cubenol-1,10-di-epi	1618	0.27
β-elemene	1389	0.28	Cubenol-1-epi	1627	0.99
α-gurjunene	1409	0.75	α-cadinol-epi	1638	1.83
Caryophyllene (E)	1417	9.22	α-muurolol	1644	0.35
Neryl acetone	1434	0.16	α-cadinol	1652	1.45
α-humulene	1452	4.28	Selin-11-en-4-α-ol	1658	1.59
Caryophyllene-9-epi (E)	1464	3.57	Citronellyl tiglate	1666	0.20
γ-muurolene	1478	0.62	Isopropyl tetradecanoate	1828	12.10
β-ionone (E)	1483	0.26	Hexadecanoic acid	1959	9.96
β-selinene	1489	0.65	Tricosane	2300	0.19
α-selinene	1498	1.30	Tetracosane	2400	0.15
Tridecanal	1509	0.43	Pentacosane	2500	1.39
γ-cadinene	1513	0.58	Hexacosane	2600	0.13
Δ-cadinene	1522	1.01	Heptacosane	2700	0.82
Calamenene-cis	1528	2.18	Nonacosane	2900	0.69
Cadina-1,4-diene-trans	1533	0.84			

The chemical composition of the essential oil of *H. italicum* is dominated by the presence of major compounds, Isopropyl tetradecanoate (12.10%), α-pinene (12.02%), hexadecanoic acid (9.96%), caryophyllene (E) (9.22%), Ledol (9.11%), palustrol (5.55%), α-humulene (4.28%), caryophyllene <9-epi> (3.75%), α-copaene (3.72%) and calamenene-cis (2.18%).

Table 3: Inhibition diameter, (mm) of *Helichrysum italicum*'s essential oil.

Microorganisms	Controls			Inhibition diameter*			
	Cefotaxine	Colistin sulfate	Gentamicine	*Dilution*			
				EO	½	1/5	1/10
Shigella sp	14	15	30	11.5	10	11.5	9
Escherichia coli ATCC 25922	33.5	15	25	14	9	9	0
Klebsiella pneumoniae	19	13	17	13	7	7	0
Staphylococcus aureus ATCC 25923	18	18	0	14	9	9	0
Pseudomonas syringae	18.5	15	22.5	14	0	0	0
Candida albicans	0	0	0	0	0	0	0

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

results show that the pure oil of *H. italicum* has moderate activity against all bacteria, producing a (0-14 mm) diameter of inhibition. While its activity is important against the bacterium *S. aureus* compared to gentamicin. The dilutions (½) have an effect on all tested bacteria, except against *P. syringae* that is resistant to all dilution. The antibiotics used in this study, as well as *H. italicum*'s oil, have no effect on the growth of the fungus *Candida albicans.* This inhibition was dependent on the sensitivity of the bacteria tested. All tested bacteria are sensitive to the antibiotics except *Staphylococcus aureus* that is resistant to the gentamicine. The *candida albicans* fungus is resistant to the antibiotics tested. The bacteriological results of *H. italicum* are similar to those in the literature. The essential oil is active against *Staphylococcus aureus* and *Echerichia coli*[29,43,44]. Other studies have showed that the extracts *from H. italicum* have the ability to inhibit the growth of *Staphylococcus* sp.[56-59]. Our investigation show a moderate inhibition of grows bacteria tested. The results showed that the oil of *H. italicum* subsp. *Numidicum* from Algeria does not look like those of the literature reporting on antifungal properties. A strong antifungal activity of *H. italicum* against *Globisporangium ultimum* is reported[38]; While the oil of this species inhibits growth of *Candida albicans*[29]. There is accordance with previous counts for this species. The observation of metaphase plates of *H. italicum* allowed us to identify a karyotype with a tetraploïde chromosome number (2n = 4x = 28); whose base number is x = 7 (Figure 4). This subspecies *numidicum*, endemic from Algeria, its distribution is very

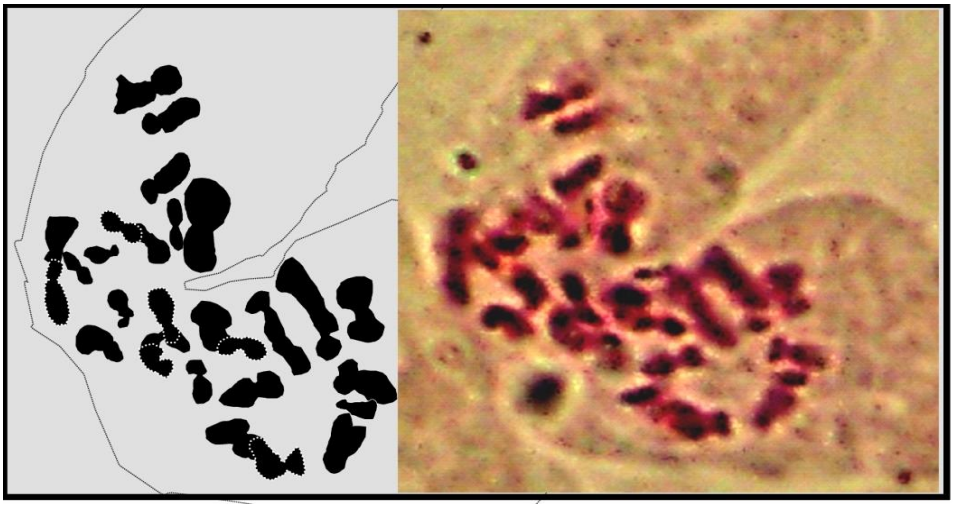

Figure 4: Caryotype of *Helichryum italicum* (2n= 4x= 28). (Magnification = HI 100X).

limited. We obtained the first chromosome number on material from Bibans (Bourdj Bouarreridj) Algeria 2n = 28. Our results agree with previous reports for material from Corsica[60] and Sardinia[52]. The same result is obtained for *H. italicum subsp. Microphyllum* from Italy[48] and Spain[50]. The same chromosome number is confirmed in *H. italicum* subsp *silculum* from Italy[45,48, 50-52].

CONCLUSION

The essential oil of *Helichrysum italicum* subsp. *numidicum*, collected from Bibans region is characterized by the main presence of Isopropyl tetradecanoate (12.1%), α-pinene (12.02%), Hexadecanoic acid (9.96%), Caryophyllene (E) (9.22%), Ledol (9.11%), Palustrol (5.55%), α-humulene (4.28%). We also note that the chemical composition of the Bibans sample differs from those reported in the literature**.** These variations may be explained by the different climates, seasons, geographic and soil conditions as well as altitude differences and harvest periods of the plant. The antibacterial activity of *H. italicum* subsp. *numidicum* 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. italicum* subsp. *numidicum* is stable and similar to bibliographic results. The chromosome count of the Bibans population is determined, and the number is tetraploïde with 2n = 4x = 28 and a basic chromosome number with x = 7.

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COMPETING INTERESTS

The authors declare that they have no competing interests

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