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Chemical composition, antibacterial activity and chromosome number of Algerian populations of two *chrysanthemum* species

Takia Lograda[1*], Messaoud Ramdani[1], Pierre Chalard[2,3], Gilles Figueredo[4], Hafsa Silini[5] and Meriem Kenoufi[1]
[1]Laboratory of Natural Resource Valorisation, Sciences Faculty, Ferhat Abbas University, 19000 Setif, Algeria. [2]Clermont Université, Université Blaise Pascal, BP 10448, F-63000 Clermont Ferrand. [3]LEXVA Analytique, 460 rue du Montant, 63110 Beaumont, France. [4]LEXVA Analytique, 460 rue du Montant, 63110 Beaumont, France. [5]Laboratory of Applied Microbiology, Sciences Faculty, Ferhat Abbas University, 19000 Setif, Algeria.

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ABSTRACT

The chemical composition of essential oil isolated from *Chrysanthemum fontanesii* and *C. coronarium* by hydrodistillation, was analysed by GC and GC/MS. A total 66 compounds representing 97.9% of the oil were identified in *C. fontanesii*, and 44 components representing 97.2% of the total oil in *C. coronarium*. The chemical composition of *C. fontanesii* and *C. coronarium*, is very different, the only common components are α-pinene, β-pinene, myrcene and Δ3-carene. The Essential oil of *C. fontanesii* and *C. coronarium* was tested for antibacterial activity against nine bacteria strains. The oil showed modest effect against *Staphylococcus aureus* ATCC 25923, and it has no significant antibacterial activity against the other bacteria tested. The population of *C. fontanesii* studied showed a diploid chromosome number 2n = 2x = 18 and a tetraploid 2n = 4x = 36 for *C. coronarium*.

INTRODUCTION

The genus *Chrysanthemum*, golden flower in Greek, belongs to the *Asteraceae* family; it includes about 300 species (Kumar *et al.*, 2005). The *chrysanthemum* is distributed in two main centres, one in the Mediterranean area, the other in China and Japan (Dowrick, 1952). In Algeria, the genus includes 20 species with 8 endemic (Quézel et Santa, 1963). *Chrysanthemum coronarium* L. is an annual herbaceous weed widely distributed in the Mediterranean region, Japan, China and The Philippines (Sanchez-Monge, 1991) and it has big capitula, usually bicolored white and yellow. The species is an ornamental plant. The variety 'spatiosum', is appreciated as a Chinese vegetable. Other uses of this species have been reported in the literature (Sulas and Caredda, 1997; Tada and Chiba, 1984; Alvarez-Castellanos and Pascual-Villalobos, 2003). *Chrysanthemum fontanesii* (B. & R.) (Q. & S.) is a perennial plant of 100-150 cm long, very rower suffrutescent at the base, the stems are not hairy. The Leaves, sessile amplexicaul are strongly toothed. The achenes are all similar, bald, without pappus. It is an endemic of North Africa, located in the Tell of Algiers and Constantine (Quézel et Santa, 1963). In Chinese medicine, *C. morifolium* is widely used as a dietary supplement or herbal tea (Chu *et al.*, 2004; Lai *et al.*, 2007) and has an antihepatotoxic and antigenotoxic effect (Lee *et al.*, 2011); it exhibit an allelopathic activity (Beninger and Hall, 2005). In Korea, China and Japan *C. indicum* is used to treat infectious diseases and disorders of hypertension (Shunying *et al.*, 2005; Lee *et al.*, 2009). It has anti-inflammatory, immunomodulatory humoral and cellular, and mononuclear phagocytic activities (Cheng *et al.*, 2005; Su *et al.*, 2011). The essential oils of *C. indicum*, *C. parthenium*, *C. trifurcatum* and *C. viscidehirtum* have a significant antibacterial activity (Ren *et al.*, 1999 ; Khallouki *et al.*, 2000 ; Shafaghat *et al.*, 2010 ; Aridogan *et al.*, 2001 ; Ben Sass *et al.*, 2008). The essential oil of *C. balsamita* is an antioxidant (Pukalskas *et al.*, 2010). The flowers of *C. cinerariaefolium* and *C. macrotum* have insecticidal and herbicide effects (Kumar *et al.*, 2005; Haouas *et al.*, 2011). *Chrysanthemum coronarium* has medicinal properties; the leaves are expectorant and stomachic, while the flowers are stomachic (Bar-Eyal *et al.*, 2006), it is used against constipation (Song *et al.*, 2003), and effective in the fight against nematodes and protects plants against caterpillars (Wood *et al.*, 2010).

* *Corresponding Author*
Email : tlograda63@yahoo.fr
Phone: (213)36835894; Fax: (213)36937943.

The extract of *C. coronarium* showed strong and selective allelopathic activity against weeds (Hosni *et al.,* 2013; Alvarez-Castellanos *et al.,* 2001). The chemical composition of essential oils of *C.coronarium* is highly variable. The major compounds are α-pinene, the camphor, β-pinene, trans-chrysanthenyl acetate, trans-chrysanthenyl isovalerate, cis-chrysanthenyl acetate, camphene and myrcene (Alvarez-Castellanos *et al.,* 2001; Senatore *et al.,* 2004; Basta *et al.,* 2007). The butanol extract of the leaves of *Chrysanthemum fantanesii* additioned with vitamin E and C reduces birth defects and oxidative damage in mice (Amrani *et al.,* 2012); it is the free radical scavengers and powerful antioxidants and has an average antibacterial activity (Ben Aissa, 2011). The phytochemical study of *C. fontanesii* unveiled biodiversity of secondary metabolites on four families': flavonoids, terpenes, phenolic acids and coumarins (Ben Aissa *et al.,* 2011). The genus *Chrysanthemum* has been many karyological studies (Abd El-Twab *et al.,* 2008 ; Lee *et al.,* 2002 ; Kondo *et al.,* 2010 ; Zhmyleva *et al.,* 2006 ; Garcia *et al.,* 2004); the chromosomes counting are focused on the Asian species, while studies on Mediterranean species are old and fragmentary or nonexistent in Algeria (Ramdani, 1993). The base chromosome number in the genus is x = 9 (Dowrick, 1952; Zhao *et al.,* 2009; Pellicer *et al.,* 2007; Inceer and Hayirlioglu-Ayaz, 2007). The *Chrysanthemum* diploid taxa are distributed mainly in Central Asia, while the tetraploid and hexaploid taxa are located in the Mediterranean, Europe, and Asia. In Japan and Taiwan the octaploid taxa and decaploide are found (Zhao *et al.,* 2009). *Chrysanthemum* shows a polyploid series of 2x to 22x, but the most species are diploid. The presence of polyploid taxa is very marked; the artioploides have a significant presence, while perissoploid are poorly represented. The highest number of chromosome was observed in *Chrysanthemum lacustre* with 2n = 198 (Dowrick, 1952; Natarajan, 1964).

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

MATERIAL & METHODS

Plant material

Chrysanthumum Fontanesii and C. coronarium are collected from two localities in eastern Algeria, Aouana (Jijel and Aouakas (Bedjaia) respectively. Aerial parts were collected during the flowering stage in June 2012. The air dried materials were subjected to hydro-distillation for 3h using a Clevenger apparatus type. Voucher specimens were deposited in the herbarium of the Department of Ecology and Biology, 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 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 (Masada, 1996; NIST, 2002) and those described by Adams, as well as on comparison of their retention indices either with those of authentic compounds or with literature values (Adams, 2001).

Antibacterial Activity

The antimicrobial activities of the essential oils were evaluated against both Gram positive (*Enterobacter cloacae* ATCC 13047, MRSA (Methicillin-resistant *Staphylococcus aureus*), *Staphylococcus aureus* ATCC 25923) and six Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas syringae, Salmonella sp, Serratia liquefaciens* ATCC 27592, *Serratia marcescens* ATCC 14756, *Shigella* sp). 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) 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 agars, 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.

Caryology

For karyotypic analysis, the squashing method is used. The root-tip meristems of from germinating seeds were usually used for chromosome preparations; only the root-tips of *C. fontanesii* were taken from wild plants in their natural habitat. 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.

Result

The hydrodistillation of the essential oil of *Chrysanthemum fontanesii* and *C. coronarium* gave a viscous liquid with a color blue and yellow, respectively. The yield of essential oil of our samples is 0.1% for *C. fontanesii* and 0.06% for *C. coronarium*. The analysis and identification of the components of the essential oil of both species was performed using the (GC-MS). The compounds identified in these oils and their relative abundances are presented in order of their appearance.

These analyses led to the identification of 66 components representing 97.9% of the total oil of *C. fontanesii* (table 1), and 44 components representing 97.2% of the total oil of *C. coronarium* (table 2). According to our results the chemical composition of the two species, *C. fontanesii* and *C. coronarium*, is very different, the only common components are α-pinene (0.3-3.33%), β-pinene (0.18-1.23%), myrcene (1.32-5.97%) and Δ3-carene (1.1-0.49%).

The chemical composition of the essential oil of *C. fontanesii* is dominated by the presence of a major product, Artemisia triene (22.3%), spatholenol (10.99%), γ-humulene (4.81%) and the trans-α-bergamotol (Z) (3.98%). We also note the presence of 24 components in concentrations greater than 1% in this oil. For the essential oil of *C. coronarium*, the major components are the 1,1-Difluror-tetramethylcyclopropane (11.52%), santolina triene (10.38%), camphre (8,89%), 2-octen-4-one, 2-methyl-1,5-heptadien-4-one,3,3,6trimethyl (8.7%), myrcene (5,97%) and lyratyl acetate (5.9%). Chamazulene which gave the blue color to the oil was present (0.95%). In addition to these major components we notice the presence of 15 compounds with a rate higher to 1%. The antibacterial activity of essential oils of *C. fontanesii* and *C. coronaruim* is evaluated by the method of disc. The screening results are expressed by measuring the diameter of the inhibition halos in mm after 24 h of incubation at 37°C (table 3). All tested bacteria are sensitive to the antibiotic gentamicin except *Enterobacter cloacae* that is resistant.

The results show that the essential oil of *C. coronarium* has low activity against *Staphylococcus aureus*, while its activity against *Salmonella* sp and *Shigella* sp is present only with pure oil, the dilutions have no effect on bacteria mentioned. The remains of the tested bacteria are resistant to *C. coronarium* oil. The concentrated essential oil of *C. fontanesii* generates moderate activity against bacteria (*Staphylococcus aureus*, *MRSA*, *Shigella* sp and *Serratia liquifaciens*). With dilutions ¼ and 1/8 only *Staphylococcus aureus* is sensitive. The rest of the bacteria are resistant to all dilution tested. Based on the results obtained, the essential oil of *C. fontanesii* has a low activity compared to the essential oil of *C. coronarium*.

The observation of metaphase plates of *Chrysanthemum fontanesii* and *C. coronarium*, allowed us to observe a diploid chromosome number 2n = 2x = 18 and a tetraploid chromosome number 2n = 4x = 36, respectively; with a basic chromosome number x = 9 (Figure 1).

Table. 1: Chemical composition of essential oil of *Chrysanthemum fontanesii*.

Compounds			**Compounds**		
Yield(v/v)	0.1	**KI**	**Yield (v/v)**	0.1	**KI**
Total	97.9		**Total**	97.9	
Nb. of compound	66		**Nb. of compound**	66	
α-pinene	0.30	931	Arteannuic alcohol <CIS->	1.39	1617
β-pinene	0.18	976	β-bisabolene	1.76	1624
6-methyl-5-hepten-2-one	0.75	983	α-santalol acetate (Z)	0.95	1630
Myrcene	1.32	987	Methyl ester of dec-4.6.8-triyn-2-	1.67	1636
Sabinene	0.35	1029	3-methyl-1-benzoxepin-5(2H)-one	1.07	1638
Citronella	0.19	1050	(-)-Sinularene	0.54	1642
α-terpinolene	0.31	1097	Caryophylla-4(12).8(13)-dien-5.bet	0.83	1647
Octen-1-ol. acetate	0.20	1104	Methyl ester of dec-4.6.8-triyn-2-	2.57	1657
Δ3-carene	1.10	1374	Cis chrysanthenol	1.38	1660
β-caryophyllene	2.95	1421	β-Eudesmol	1.16	1666
Artemisia triene	22.30	1452	Cis γ-bisabolene	0.21	1679
Trans β-farnesene	0.29	1457	Caryophyllenol-II	1.06	1685
α-humulene	0.25	1477	Cyclobuta[1.2:3.4]dicyclooctene	0.56	1688
Germacrene-D	1.03	1482	β-bisabolenol	1.70	1692
γ-humulene	4.81	1487	Cis mentha-1(7).8-dien-2-ol	0.63	1696
α-farnesene (Z.E)	0.28	1496	(+)-β-Costol	0.41	1701
β-sinensal	1.48	1501	4.4-dimethyltricyclo[6.3.2.0(2.5)]	0.28	1738
α-farnesene (E.E)	0.35	1516	7-exo-T-butyl-3-oxabicyclo-(3.3.1)	0.67	1754
α-Cubebene	0.36	1520	4.4-Dimethyltricyclo[6.3.2.0(2.5)]	0.56	1770
Linalool-dehydro	1.65	1526	Isopulegyl acetate <neoiso->	0.17	1784
β-ocimene (Z)	0.58	1544	10-epi-cubebol	0.20	1793
Arthole	0.68	1547	Trans limonene oxide	0.27	1802
Farnesol <Z. E>	0.58	1552	Nerolidol	1.18	1854
(7S)trans-Bicyclo (4.3.0)-3-nonen-7	2.36	1556	β-ionone	1.05	1922
Cis α-santalol	1.63	1558	2.8.10-pentadecatriene-4.6-diyne	2.54	1957
Δ-Nerolidol	1.38	1561	1.2-benzenedicarboxylic acid. buty	0.45	1992
Santolina triene	0.81	1565	2.2-diphenylethylamine	0.35	2078
Iso longifolol	0.89	1567	5-(2-methylbutyl)-2-ethoxy-3-meth	0.83	2088
Perillaldehyde	1.88	1579	2-ethynyl-bicyclo[4.4.1] undeca-1.3	0.97	2102
Spathulenol	10.99	1585	Phytol	1.10	2130
Caryophyllene oxide	0.61	1594	1H-phenalene	0.18	2155
β-sinensal	1.61	1596	2-phenyl-D(5)-thiophene	0.53	2176
Trans α-bergamotol (Z)	3.98	1610	n-Tricosane	0.27	2494

Table. 2: Chemical composition of essential oil of Chrysanthemum coronarium.

Compounds			**Compounds**		
Yield (v/v)	**0.07**	**KI**	**Yield (v/v)**	**0.07**	**KI**
Total	**97.2**		**Total**	**97.2**	
Nb. of compound	**44**		**Nb. of compound**	**44**	
Santolina Triène	10.38	908	2-Octen-4-one. 2-methyl-1.5-Heptadien-4-one. 3.3.6-trimethyl	8.7	1505
Tricyclene	0.16	926	Δ-cadinene	2.24	1520
α-pinene	3.33	931	Hexane. 3-ethyl-	0.44	1524
Camphene	2.03	947	Benzene. 1-ethyl-3.5-dimethyl-	0.71	1540
β-pinene	1.23	972	Valerate de myrtenyle	0.13	1614
Myrcene	5.97	992	1-Methyl-1-silabenzocyclobutene	3.73	1617
Δ3-carene	0.49	1011	Dill apiole	1.6	1622
Limonene	1.43	1031	Epi-cubenol-1	3.35	1627
β-ocimene (E)	0.43	1050	Benzene. 4-ethyl-1.2-dimethyl-	1.47	1630
7-methylenebicyclo[4.2.0]octane	1.84	1090	1.4-Ethenonaphthalene-1-4-di-oH	1.89	1635
Camphre	8.89	1144	1.1-Difluror-tetramethylcyclopropane	11.52	1640
Borneol	0.62	1166	Chamazulène	0.95	1719
Hexanal. 3.3-dimethyl-	1.05	1195	Myristic acid	0.71	1764
Trans chrysantenyle acetate	2.26	1230	2-Pentadecanone-6.10.14-trimethyl	0.53	1837
Lyratyl acetate	5.9	1260	2-hexa-2.4diyn-1-yl iden-1.6-dioxaspiro [4.4]non-3-ene	0.89	1878
Acetate de bornyle	2.13	1287	Hexadecanoic acid MERCK	0.89	1987
Capric acid	0.55	1370	Abietatriene	0.11	2054
α-copaene	0.33	1379	Nonahexacontanoic acid	0.19	2110
Italicene	1.9	1410	Linoleic acid	0.4	2156
Iso bazzanene	0.16	1442	n C23H48	0.54	2300
Trans β-bergamotene	2.17	1480	Docosane. 7-hexyl-	0.58	2350
β-selinene	2.06	1485	n C25H52	0.27	2500

Table. 3: Antibacterial activity of *Chrysanthemum fontanesii* and *C. Coronarium.*

Bacteria	**A**	***C. fontanesii***				***C. coraonarium***			
		EO	***Dilution***			**EO**	***Dilution***		
			1/2	**1/4**	**1/8**		**1/2**	**1/4**	**1/8**
Staphylococcus aureus ATCC 25923	25	15	0	7	8	9	9	7	10
Shigella sp	15	8	0	0	0	10	0	0	0
Serratia liquifaciens	10	8	0	0	0	0	0	0	0
MRSA	14	8	0	0	0	0	0	0	0
Salmonella sp	16	0	0	0	0	10	0	0	0
Serratia marcescens	12	0	0	0	0	0	0	0	0
Echerichia coli ATCC 25923	14	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa ATCC 27853	10	0	0	0	0	0	0	0	0
Enterobacter cloacae	0	0	0	0	0	0	0	0	0

A: Gentamicine; EO: Essential oil; *MRSA*: Methicillin-resistant *Staphylococcus aureus*

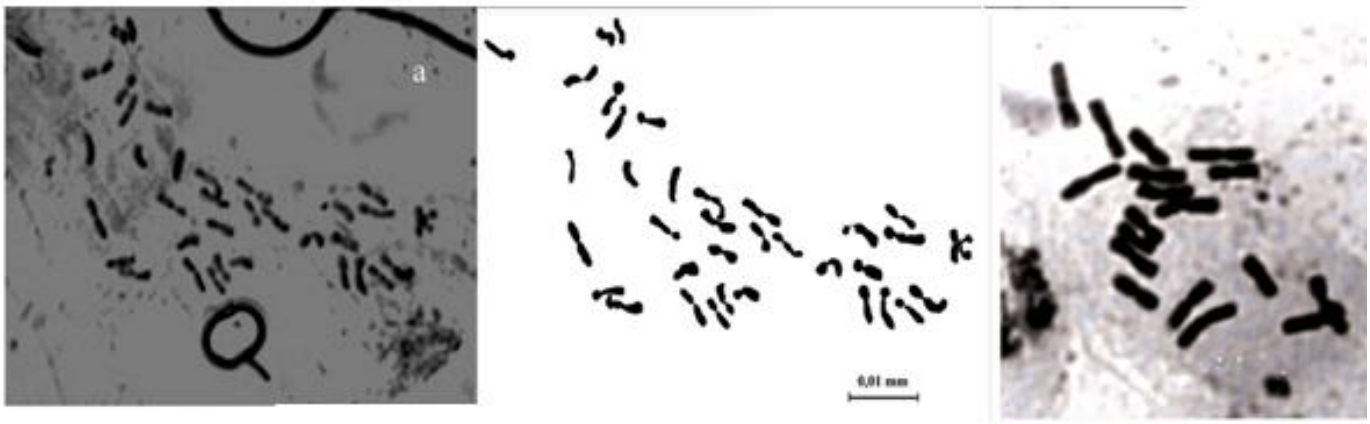

Fig. 1: Somatic chromosomes of *Chrysanthemum* (magnification = HI 100X). (a) *Chrysanthemum coronarium* (2n= 4x= 36) (b) *Chrysanthemum fontanesii* (2n = 2x= 18).

DISCUSSION

Generally yields essential oil of the genus Chrysanthemum are low. The oil yield was 0.055% for *C. trifurcatum* (Ben Sass *et al.,* 2008) and 0.23% for *C. indicum* (Shunying *et al.,* 2005). Compared with other herbs, (1 to 2.5%) for *Rosmarinus* and (2 to 2.75%) for *Thymus* (Edward *et al.,* 1987), the yield of *C. fontanesii* and *C. coronarium* are very low.

The essential oils of *C. coronarium* from Greece contain the major compounds, the Camphor, trans-Chrysanthenyl acetate, cis-Chrysanthenyl acetate and the β-pinene oxide (Basta *et al.,* 2007). The oils of *C. coronarium* collected in the regions of Italy have almost the same major compounds (Trans-Tonghaosu, trans-chrysanthenyl acetate, cis-chrysanthenyl and camphor) (Senatore *et al.,* 2004).

The chemical profile of the essential oil of *C. coronarium* (of the region Aouakas, Algeria) is different from those reported by other authors. Our sample contains high percentages of 1,1-Difluror-tetramethylcyclopropane, camphor and santolina triene, these components are absent in populations of Greece and Italy, while the trans-chrysanthenyl acetate, cis-chrysanthenyl acetate, the β-Pinene oxide and trans-Tonghaosu are absent in the sample of Algeria.

The bacteriological results of *C. coronarium* are similar to those in the literature. The essential oils of *C. coronarium* of Italy have no activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Senatore *et al*., 2004).

Our karyological result of *C. coronarium* is similar to those found by (Dowrick, 1952) with 2n = 36, and also reported a diploid with 2n = 18 in the Mediterranean. The Turkey and Egypt populations of *C. coronarium* have diploid with 2n = 18 (Abd El-Twab *et al.,* 2008; Inceer and Hayirlioglu-Ayaz, 2007). The same result with 2n = 2x = 18 is reported by (Ramdani, 1993) in the population of Guerrouch (Jijel) for *C. fontanesii.* The basic chromosome number of *C. coronarium* and *C. fontanesii* is x = 9.

This number is most common in the genus *Chrysanthemum*, in the tribe *Anthemideae* well as in the family *Asteraceae*, it is the ancestral basic number (Bremer, 1994). The diploid and tetraploid taxa of the *Chrysanthemum* are distributed mainly in Mediterranean and Europe (Dowrick, 1952), which is consistent with our results.

CONCLUSION

The results of the analysis show that the chemical composition of essential oils of *C. coronarium* differs from those of *C. fontanesii*. The testing of the antibacterial activity of essential oils of *C. coronarium* and *C. fontanesii* by the method of record shows that the oils of both species have a very low antibacterial activity. The karyological study of two species, *C. coronarium* and *C. fontanesii* , based on chromosome counting, allows us to determine two chromosome numbers, a tetraploid with 2n = 4x = 36 for *C. coronarium* and a diploid with 2n = 2x = 18 for *C. fontanesii*, with a basic chromosome number x = 9.

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