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Original Research Article

Chemical composition, Antimicrobial activity and chromosome number of *Globularia alipum* from Algeria

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A B S T R A C T

The chemical composition of essential oil, isolated from *Globularia alipum* by hydrodistillation, was analysed by GC and GC/MS. A total 39 compounds representing 98.9% of the oil were identified in *Boutaleb population*, and 89.7% of the total oil in Khenchela population. The essential oil of *G. alipum* is Characterized by a high rate of hexadecanoic acid (palmetic acid), 14.64% for Boutaleb population and a rate of 29.52% for Khenchela population. Other major compounds are present in the essential oils of *G. alipum*, the phytol isomer (9.9-5.43%); (Z.Z)-6,9-cis-3,4-epoxy-nonadecadi (8.27-5.45%); 1,2-Benzene dicarboxylic acid-bis (4.68-6.09%); L-linalool (3.49-3.83%) and the heptadecane (2.29-3.28%) respectively for Boutaleb and Khenchela populations. To test the antibacterial activity of essential oil of *G. alipum*, eleven bacteria are used in this study. The oil showed a significant effect against Gram-negative bacteria, and modest antibacterial activity against Gram-positive bacteria. The populations of *G. alipum* studied showed a diploid chromosome number with 2n = 2x = 16, and a basic chromosome number x = 8.

Keywords

Globularia alipum, Essential oil, antibacterial activity, Chromosome, Algeria

Introduction

The genus *Globularia* (Family: *Globulariaceae*) consists of plants which are herbs, chamaephytes or shrubs, common in the Mediterranean regions, Europe and North Africa. It includes one species in Algeria, *Globularia alipum* L., with two sub-species.

The ssp. *eu-alypum* L. is common to all Algeria, while the subspecies *arabica* (Jaub. and Spach) Mayor (synonym: *G. vesceritensis* Batt and *G. eriocephala* Pomel.) is confined to the Sahara (Quézel et Santa, 1963).

Most important chemical investigations of *G. alypum* are those of (Chaudhuri and Sticher, 1981; Ben Hassine *et al*. 1982; Es Safi *et al*., 2005, 2006, 2007; Boutiti *et al*., 2008). The chemical composition show the presence in *G. alipum* the phenolic acid (Ben Hassine *et al.,* 1982; Djeridane *et al.,* 2006) and the iridoid glucosides (Chandhuri and Sticher, 1981; Es-Safi *et al*., 2006). The iridoid glycosides were isolated from *G. dumulosa* and *G. davisiana* by Hasan *et al.,* (2003) and Calis *et al.,* (2002) respectively.

The extract of *G. alypum* is used as a source of potential antioxidants (Es-Safi *et al.,* 2005; *Djeridane et al.,* 2006, 2010; Ben Mansour *et al.,* 2012). However, different extracts of *Globularia alypum* were significant source of compounds with antioxidant, antigenotoxic and anti-tuberculosis activities (Khlifi *et al*., 2005, 2011; Harzallah *et al*., 2010). *G. alypum* leaves Infusion is Toxic (Bellakhdar., 1997), hypoglycemic (Ziyyat *et al.,* 1997; Skim *et al*., 1999; Bellakhdar *et al.,* 1991; Zennaki *et al*., 2009; *Boutiti et al*., 2008; Taleb-Dida and Bouchenak, 2011), laxative, cholagogue, stomach disorders, sudorific, purgative (Bellakhdar *et al.,* 1991; Sijelmassi, 1993; Allali *et al*., 2008; Baba Aissa, 1999), Antidiabétic (Bnouham *et al.,* 2002). The leaves and stems of *G. alipum* are used as decoction in the treatment of hypoglycemia, rheumatic and infectious diseases, its leaves are reported to be used in the treatment of diabetes, in renal and cardiovascular diseases (Jouad *et al.,* 2001, 2002; Ziyyat *et al.,* 1997; Merghache *et al*., 2013).

In addition, *G. alypum* was shown to exert an anti-ulcer activity (Fehri and Aiache, 2010). *G. alypum* is effective by lowering lipid peroxidation and improves antioxidant enzymes (Taleb-Dida and bouchenak, 2011). The decoction prepared from the leaves can also be drank with some honey to treat digestive troubles including stomach and intestinal pains, high blood pressure, heart disorders, renal colic and diabetes (Ben Hji *et al*., 2007, 2011).

G. alipum is traditionally used in North Africa especially in Algeria as folk medicine in the treatment of many illnesses. In Morocco, the leaves of *G. alipum* are used in traditional medicine as hypoglycaemic, a laxative, a purgative, a myorelaxant and an antispasmodic remedy (Ziyyat *et al.,* 1997; Merzouki *et al.,* 2000; Jouad *et al.,* 2002; Chokri *et al*., 2010). *G. alypum* extract showed an important anti-inflammatory activity (Khlifi *et al*., 2013) and it is a hypoglycaemic (Skim *et al.,* 1999; Mansar-Benhamza *et al.,* 2013). Somme species of *Globularia* have a good antimicrobial activity on tested bacteria (Meddah *et al.,* 2011). *Pseudomonas aeruginosa* was highly resistant to some antibitics and it was sensitive to extracts of *G. alypum* (Bouabdelli *et al.,* 2012).

The species of the genus *Globularia* studied have shown a diploid chromosome number, 2n = 16, *G. alypum* (Nilsson and Lassen, 1971; Sales and Hedge, 2013), *G. repens* 2n = 16 (Contandriopoulos, 1978; Sales and Hedge, 2013) and *G. amygdalifolia* 2n = 16 (Liv, 1980). Sales and Hedge, (2013) reported a diploid chromosome number 2n = 16 for (*G. bisnagaria*, *G. fuxeensis*, *G. nudicaulis* and *G. spinosa*), and a tetraploid number 2n = 32 for *G. cordifolia*, *G. vulgaris*. The karyotype of *G. majoricensis* shows a tetraploid with 2n = 32 (Contandriopoulos, 1978; Cardona and Contandriopoulos, 1980; Mercedes and Rossello, 2006; Sales and Hedge, 2013). Sales and Hedge, (2013) identified a hexaploid chromosome number 2n = 8x =64 for *G. majoricensis*, while Schwartz, (1963) reported for this species an aneuploid with

2n = 62. The chromosome number of *G. incanescens*, *G. trichosantha*, and *G. punctata* is reported to 2n = 16 (Flora Europea, 1982).

To the best of our knowledge, the chemical composition of essential oil of *Globularia alipum* has not been studied yet. The aim of this work was to investigate the chemical composition, antibacterial activity of essential oil and chromosome numbers from *G. alipum* growing in Algeria.

Materials and Methods

Plant material

Globularia alipum is collected from two localities in eastern Algeria, Boutaleb (Setif) and Khenchela. Aerial parts were collected during the flowering stage in April 2014. 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 Extract Essential oil was tested against the following bacteria; seven gram negative bacteria: *Acinetobacter baumanii* ATCC 19606; *Citrobacter freundii* ATCC 8090; *Escherichia coli* ATCC 25922; *Salmonella typhimurium* ATCC 13311; *Klebsiella pneumoniae* ATCC 700603; *Proteus mirabilis* ATCC 35659; *pseudomonas aeruginosa* ATCC 27853, and five gram positive bacteria; *Bacillus cereus* ATCC 10876; *Bacillus subtilus* ATCC 663313; *Enterococcus faecalis* ATCC 49452; *Lysteria monocytogenes* ATCC 15313 and *Staphylococcus aureus* ATCC 25923. The *in vitro* antibacterial activity of the examined extract was assessed the determination of the activity by the micro dilution method, according to recommendations of the Clinical and Laboratory Standards Institute.

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. A pre-treatment at room temperature for 1.15 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 and Discussion

The essential oil, of two localities of *Globularia alipum*, isolated by hydrodistillation from the aerial parts, was obtained in yield of 0.08 and 0.06% (v/w). The chemical composition of essential oil, analyzed by gas chromatography/mass spectrometry (GC-MS), gave 30 constituents representing 98.93% of the total oil of Boutaleb population and 30 compounds representing 89.73% of the total oil of Khenchela population. The names of the corresponding compounds and their percentages are listed in table 1.

The essential oil of *G. alipum* is Characterized by a high rate of hexadecanoic acid (palmetic acid), 14.64% for the Boutaleb population and a rate of 29.52% for Khenchela population. Other major compounds are present in essential oils in both populations, the phytol isomer (9.9-5.43%); (Z.Z)-6,9-cis-3,4-epoxy-nonadecadi (8.27-5.45%); 1,2-benzenedicarboxylic acid-bis (4.68-6.09%); L-linalool (3.49-3.83%) and the heptadecane (2.29-3.28%) respectively for Boutaleb and Khenchela population.

The chemical composition of the two populations differ considerably, the Boutaleb population is rich in (1-octen-3-ol; L-camphor; α-terpineol; cis-3-hexenyl-tiglate; eugenol; docosane; nerolidol <E->; Cis-3-hexenyl-benzoate and α-bisabolol), which are poorly represented in the population of Khenchela, whereas Khenchela population is characterized by (the 2-pentadecanone 6,10,14-trimethyl; germacrene-B; 8-octadecenoic acid-methyl ester; nonadecane and β-caryophyllene), which are feebly represented in the population of Boutaleb.

The results of the experiments assessing the bacteriostatic effects of *G. alipum* essential oil of the study on Gram-negative and Gram-positive bacteria *Acinetobacter baumanii* ATCC 19606; *Citrobacter freundii* ATCC 8090; *Escherichia coli* ATCC 25922; *Salmonella typhimurium* ATCC 13311; *Klebsiella pneumoniae* ATCC 700603; *Proteus; Bacillus subtilus* ATCC 663313; *Enterococcus faecalis* ATCC 49452; *Lysteria monocytogenes* ATCC 15313 and *Staphylococcus aureus* ATCC 25923 are presented in (Table 2). Effectively, the essential oil from *G. alipum* leaves were demonstrated antibacterial activity against all clinical pathogens bacteria tested.

The essential oil of Boutaleb and Khenchela populations showed important activities against gram negative bacteria and the moderate activities against *Acinetobacter baumanii*, while the activity is low to absent against *Klebsiella pneumoniae* and *Proteus mirabilis* (Figure 1). The antibacterial activity of the essential oil of *Globularia alipum* is significant against the Gram-positive bacteria. *Bacillus cereus* and *Staphylococcus aureus* show a resistance to essential oil of this species (Figure 2). The bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Proteus mirabilis* are resistant to essential oils of the populations studied. The oil of Khenchela population has no effect on *Proteus mirabilis* and *Staphylococcus aureus* bacteria.

The observation of metaphase plates of *Globularia alipum*, allowed us to observe a diploid chromosome number 2n = 2x = 16 in Boutaleb and Khenchela populations with a basic chromosome number x = 8 (Figure 3).

Generally, the yields average essential oil of the *Globularia alipum* is very low (0.07%), compared with other herbs is (0.075%) for Chrysanthemum (Lograda *et al.,* 2013), (1 to 2.5%) for *Rosmarinus* and (2 to 2.75%) for *Thymus* (Edward *et al.,* 1987). Attia *et al.,* (2001) give a yield of 0.01% for *G. alipum*.
The extracts study of *G. alypum* essential oil showed significant antibacterial activity (Oran and Raies, 2000; Sultan and Zaki, 2009). The essential oil of *G. alypum* has a strong activity against *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, the same findings were observed by Bouabdelli *et al.,* (2012). The observations confirm the antibacterial effectiveness of *G. alipum* oil on *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Escherichia coli* and *Staphylococcus aureus*, as it was reported by Meddah *et al.,* (2011).

The essential oil of *G. alypum* inhibits the growth of *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *Acinetobacter baumanii*, *Citrobacter freundii*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus* and *Enterococcus faecalis*, the same effects were observed on same bacteria under the effect of the methanolic extract of *G. alypum* (Naoual *et al.,* 2014).

The chromosome number of the *Globularia* genus is 2n = 16. The study of *G. alypum* shows a diploid chromosome number 2n = 16 (Nilsson and Lassen, 1971; Sales and Hedge, 2013). The same chromosome number was observed in *G. repens* (2n = 16) (Contandriopoulos, 1978; Sales and Hedge, 2013) and in *G. amygdalifolia* (Liv, 1980). Sales and Hedge, (2013), report a diploid chromosome number of 2n = 16 for *G. bisnagaria*, *G. fuxeensis*, *G. nudicaulis* and *G. spinosa*); and a tetraploid chromosome number 2n = 32 for *G. cordifolia* and *G.vulgaris*.

The karyotype of *G. majoricensis* shows a tetraploid with 2n = 32 (Contandriopoulos, 1978; Cardona and Contandriopoulos, 1980; Mercedes and Rossello, 2006; Sales and Hedge, 2013), for this species, Sales and Hedge, (2013) identify a heptaploide chromosome number 2n = 8x = 64, while Schwartz, (1963) reported for the same species an aneuploide with 2n = 62. The chromosome numbers of *G. incanescens*, *G. trichosantha* and *G. punctata* are reported to 2n = 16 (Flora Europea, 1982) and 2n = 16 for *Globularia cordifolia* (Kuzmanov and Jurukova, 1977; Kuzmanov 1993).

Figure.1 Antibacterial activity of *Globularia alipum* (Boutaleb population)

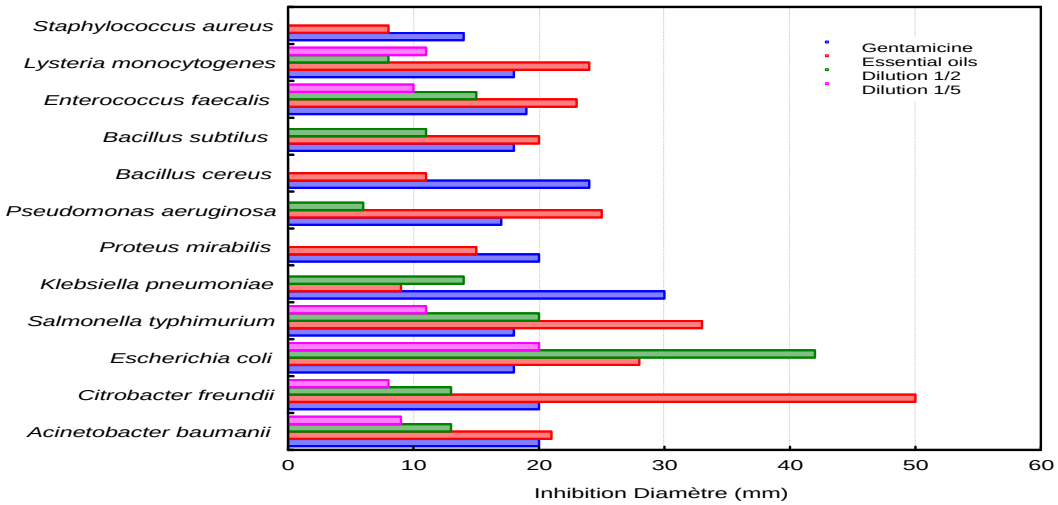

Figure.2 Antibacterial activity of *Globularia alipum* (Khenchela population)

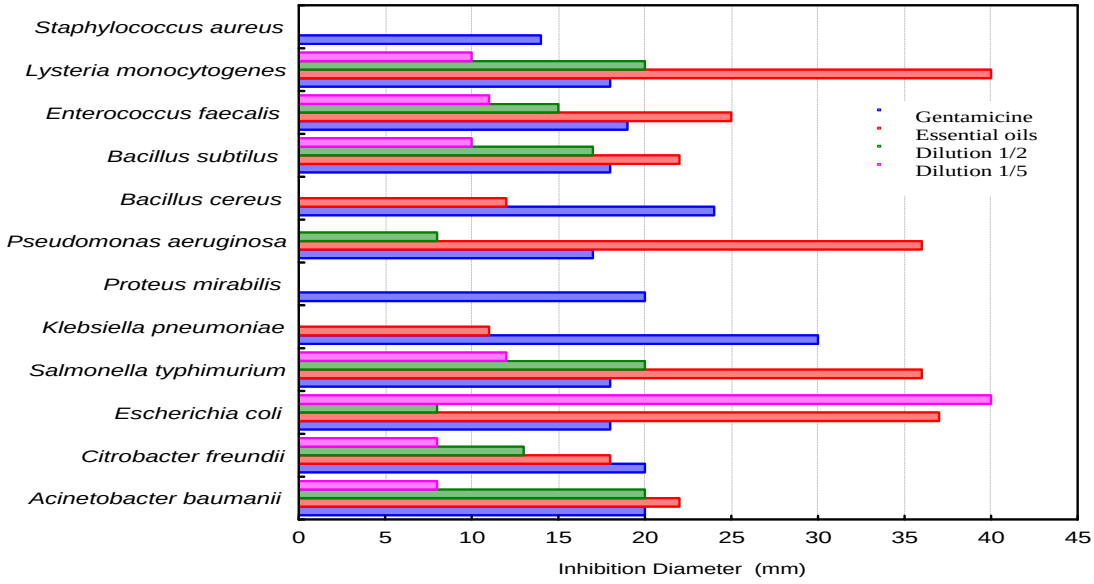

Table.1 Chemical composition of essential oils of *Globularia alipum*

	KI	Boutaleb	Khenchela
Yield (v/v)		0.08	0.06
Number of compounds		39	39
Total		98.93	89.73
Camphene	948	0.36	0.27
1-octen-3-ol	979	10.32	0.82
L-linalool	1098	3.49	3.83
L-camphor	1148	2.27	0.30
α-terpineol	1195	4.53	0.18
Cis-3-hexenyl α-methylbutyrat	1228	1.28	1.07
Geraniol	1248	0.61	0.23
4-vinyl-2-methoxy-phenol	1308	0.36	0.77
Cis-3-hexenyl tiglate	1320	2.12	0.20
Eugenol	1350	3.06	0.22
β-damascenone	1378	0.63	0.20
β-caryophyllene	1420	0.58	2.57
Geranyl acetone	1444	0.47	0.24
β-farnesene-trans	1449	1.34	0.26
β-ionone	1477	0.55	1.25
Nerolidol <E->	1557	2.64	0.24
Cis-3-hexenyl benzoate	1570	4.19	0.32
Benzoic acid. hexyl ester	1577	0.46	0.24
5-methylene-6-hepten-3-ol	1613	0.42	0.32
Epizonarene	1684	0.65	0.43
α-bisabolol	1755	4.51	1.75
Zerumbone	1778	0.75	0.45
Tetradecanoic acid (Myristic acid)	1812	0.16	0.22
2-Pentadecanone 6,10,14-trimethyl	1836	1.30	5.41
1,2-Benzenedicarboxylic acid. Bis	1854	4.68	6.09
Hexadecanoic acid. methyl ester	1920	0.76	0.33
1,2-Benzenedicarboxylic acid. Dibu	1949	1.47	0.38
Hexadecanoic acid (Palmetic acid)	1957	14.64	29.52
Germacrene-B	2047	1.84	3.87
8-Octadecenoic acid, methyl ester	2094	0.74	6.01
Phytol isomer	2102	9.90	5.43
(Z.Z)-6,9-cis-3,4-epoxy-nonadecadi	2130	8.27	5.45
Nonadecane	2293	0.89	3.21
Tetracosane	2393	0.51	0.27
Heptadecane	2494	2.29	3.28
Hexacosane	2593	0.60	0.21
Docosane	2668	3.81	1.61
Eicosane	2752	0.85	1.67
Nonacosane	2787	0.63	0.60

Figure.3 Somatic chromosomes of *Globularia alipum* (magnification = HI 100X).
(a) Boutaleb population (2n= 2x= 16) ; (b) Khenchela population (2n = 2x= 16).

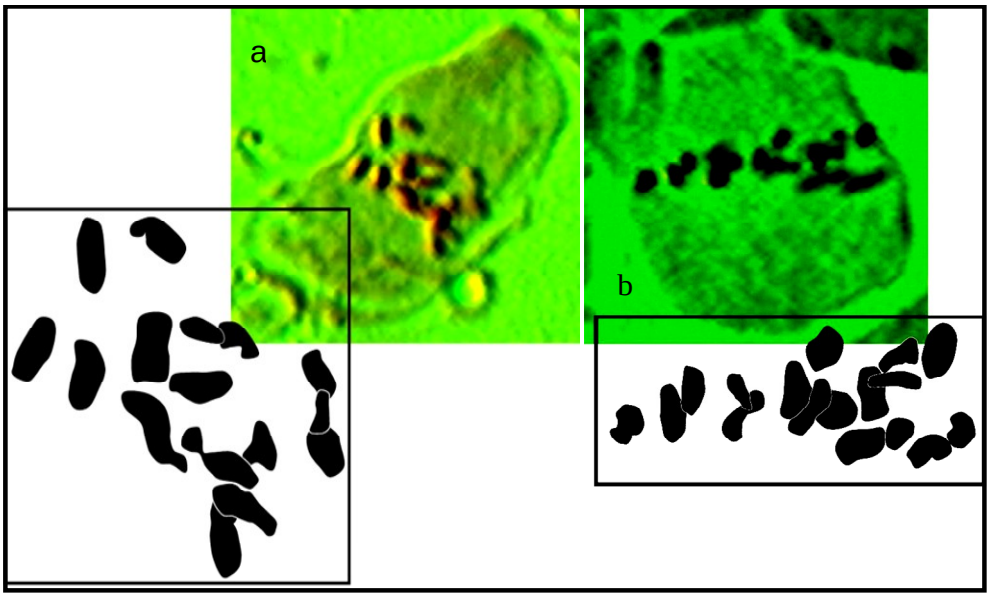

Table.2 Antibacterial activity of *Globularia alipum*

Bacteria	Population	Boutaleb			Khenchela		
	Gentamicine	*Dilution*					
		1	1/2	1/5	1	1/2	1/5
Acinetobacter baumanii ATCC 19606	20	21	13	9	22	20	8
Citrobacter freundii ATCC 8090	20	50	13	8	18	13	8
Escherichia coli ATCC 25922	18	28	42	20	37	8	40
Salmonella typhimurium ATCC 13311	18	33	20	11	36	20	12
Klebsiella pneumoniae ATCC 700603	30	9	14	0	11	0	0
Proteus mirabilis ATCC 35659	20	15	0	0	0	0	0
Pseudomonas aeruginosa ATCC 27853	17	25	6	0	36	8	0
Bacillus cereus ATCC 10876	24	11	0	0	12	0	0
Bacillus subtilus ATCC 663313	18	20	11	0	22	17	10
Enterococcus faecalis ATCC 49452	19	23	15	10	25	15	11
Lysteria monocytogenes ATCC 15313	18	24	8	11	40	20	10
Staphylococcus aureus ATCC 25923	14	8	0	0	0	0	0

The karyotype of this species comprises small chromosomes. Our results agree with previous counts by Nilsson and Lassen, (1971) and Sales and Hedge, (2013). The basic chromosome number of *G. alipum* is x = 8. This number is most common in the genus *Globularia* well as in the family *Globulariaceae*, it is the ancestral basic number.

The analysis of the chemical composition of the essential oil *Globularia alypum* by GC/MS has allowed the identification of 39 compounds. The palmitic acid is the major component of the chemical composition, although the study has identified the other natural products as minor components.

The antibacterial activity of *Globularia alypum* essential oils is tested on 12 bacterial strains. The results show that the essential oils of this species have significant inhibitory action on almost all the bacteria tested. The extracts leaves from *Globularia alypum* may be a promising alternative treatment of localized infections even with severe hospital acquired strains. Further investigations must be made to determine the active constituent(s) for their application in medical research. The chromosome number of *Globularia alypum* is stable and similar to bibliographic results. The chromosome count of *G. alypum* of the Boutaleb and Khenchla populations are determined, and the number is diploid with 2n = 2x = 16 and a basic chromosome number with x = 8.

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