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Chemical composition, antimicrobial activity and chromosome number of *Hertia cheirifolia* L. from Algeria

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Abstract: *The aims of this work are to investigate the chemical composition, the antibacterial activity of the essential oil and the chromosome numbers of two populations of Hertia cheirifolia. The samples were collected in the flowering stage, in eastern Algeria locality. The aerial parts of H. cheirifolia were submitted to a hydro-distillation. GC and GC / MS analysed the chemical compositions of the obtained essential oils. The antibacterial activity of essential oils was evaluated using the disks diffusion method against ten bacterial strains. For karyotypic analysis, the squashing method is used. Fifty-eight compounds representing 98.93% of the total oil were identified in H. cheirifolia. The chemical composition is dominated by the presence of major products, α-pinene (48.49 - 53.85%) and Germacrene-D (2.64 - 12.66%). Two distinct chemical breeds were identified, the α-pinene-spathulenol of Batna population, and the α-pinene-germacrene-D of Setif population. The essential oil of H. cheirifolia has a moderate activity against bacteria tested. In contrast, the strains E. coli ATCC 25922, P. syringae ATCC 53543 and E. fecalis ATCC 49452 are resistant to H. cheirifolia essential oils. The observations of root cells meristematic at metaphase of H. cheirifolia gave a diploid chromosome number 2n = 2x = 20, with a basic chromosome number (x= 10).*

Keywords: *Hertia cheirifolia*, essential oil, antibacterial activity, chromosome number, Algeria

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Introduction

Hertia (*Asteraceae*) includes 12 species distributed in Africa and Asia [1-3]. The genus is represented by *Hertia cheirifolia* L. species in Algeria and Tunisia [4]. *H. cheirifolia* is a perennial, glabrous, dense clump, with stems lying at the base and then ascending. The leaves are alternate, sessile, somewhat fleshy, oblong-mucronate and entire. The lemon-yellow flower heads are solitary on bare pedicels and widened at the top [4]. This plant has two synonyms, *Othonnopsis cheirifolia* (L.) Batt. & Trab. and *Othonna cheirifolia* L.

In South Africa, *H. cheirifolia* is used to treat infections of the digestive system, indigestion, diarrhea and flatulence [5, 6]. *H. cheirifolia* can provide valuable acaricide activity [7]. In Tunisia it is known as a medicinal plant with several pharmaceutical and biological activities, it reduces hyperglycemia and treats rheumatic pains [8].

Studies of *H. cheirifolia* essential oil have shown that they are different from those of genus *Hertia* species. The analysis of the essential oils obtained from *H. maroccana* revealed the presence of germanicol (17.8%), β-pinene (14.6%), α-guaiene (5.8%) and germacrene-D (5.6%) as main components [9]. *H. angustifolia* has β-pinene (51.5%), β-phellandrene 16.5% and α-pinene (13.9%) as major constituents [10]. *H. intermedia* contain five major components, β-pinene, α-pinene, α-thujene, β-phellandrene, and germacrene-D [11]. The methanolic and chloroformic extracts of the aerial parts of *H. pallens* contain a large amount of sesquiterpenes [1]. The methanolic extract of *H. cheirifolia* has significant antioxidant activity [12-16].

Chemical studies of *H. cheirifolia* extracts revealed the presence of eremophilenolides and steroids [17-19]. The extracts of the leaves of *H. cheirifolia* contain polyphenols and flavonoids [12]. These extracts have important pharmacological properties [20]. The *H. cheirifolia* extracts have antispasmodic and anti-inflammatory activities [19], acaricide [21] and anti-fumigant activities [22].

Previous studies of *H. cheirifolia* essential oil showed the presence of monoterpenes and sesquiterpene compounds [14]. Zellagui et al. [23] found the drimenin was the main constituent of the essential oil of *H. cheirifolia;* however, the study of Segueni et al. [15] showed that the majorities' compounds were monoethyl-hexyl phthalate and valeranone.

The Tunisia populations of *H. cheirifolia* contain thymol as the major component [7], while another study on Tunisian populations has showed the presence of thymol, 2,6-dimethoxy-phenol, camphor and terpinene-4-ol as majors components [21]. The studies of Majouli et al. [24] showed the presence of β-pinene, β-phellandrene and α-pinene as major components, while that of Boulechfar [25] shows the presence of α-pinene and 2-(1-cyclopent-1-enyl-1-methylethyl)-cyclopentanone. The oil of Oum Elbouaghi (Algeria) population shows the presence of monoethyl-hexyl phthalate, valeranone, drimenin [15].

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DOI: 10.2478/asn-2020-0018

The methanolic extracts of *H. cheirifolia* have moderate antibacterial activity [15-16, 26-27], whereas Bousselsela et al. [13] reports that oils of this species are ineffective against most bacterial strains tested. The essential oil of *H. maroccana* is considered moderately active against gram-negative and those of gram-positive [9]. Chromosome counts of *H. cheirifolia* are rare. Chromosome counting of Tunisian populations gave a chromosome number of 2n = 20 [28].

Therefore, the purpose of the present study was to determine the chemical composition and to evaluate the antibacterial activities of two populations of *H. chierifolia* essential oils, from eastern Algeria (Batna and Setif), as new potential source of natural antibiotic components, and determine the chromosomal number of this species.

Material and Methods

Plant materials

Samples of *H. cheirifolia* were collected at the flowering stage of two localities in eastern Algeria. Setif is the first station, with a semi-arid climate, cold in winter and warm in summer. The average annual rainfall in the Setif region is 322 mm and the average annual temperature is 15°C. The second station is Batna with a climate semi-arid to steppe type. The annual average rainfall is 210 mm; the average annual temperature is 14.2°C (Figure 1). Lograda T., Professor of Botany at the Setif-1 University, carries out the identification of samples.

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-1 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. The yield based on the dried weight of the samples was calculated using the relationship.

$$\text{Yield in essential oil (\%)} \frac{\text{weight of the extracted essential oil (g)}}{\text{weight of the plant treated in (g)}} x100$$

Essential oil analysis

The essential oils were analyzed on a Hewlett-Packard gas chromatograph CPG/FID 7890, coupled to a gas chromatograph: CPG/MS 7890/5975C, equipped with a Column Apolar: DB5 MS: 40 m 0.18 mm 0.18 µm, programming from 50°C for 5min – 5°C/min until 300°C. Helium was used as the carrier gas (1.0 ml/min); injection in split mode (1:30), injector and detector temperature is 280°C with split 1/100. 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

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on the comparison of their mass spectra with those of NIST mass spectral library [29-30] and those described by Adams as well as on comparison of their retention indices either with those of authentic compounds or with literature values [31].

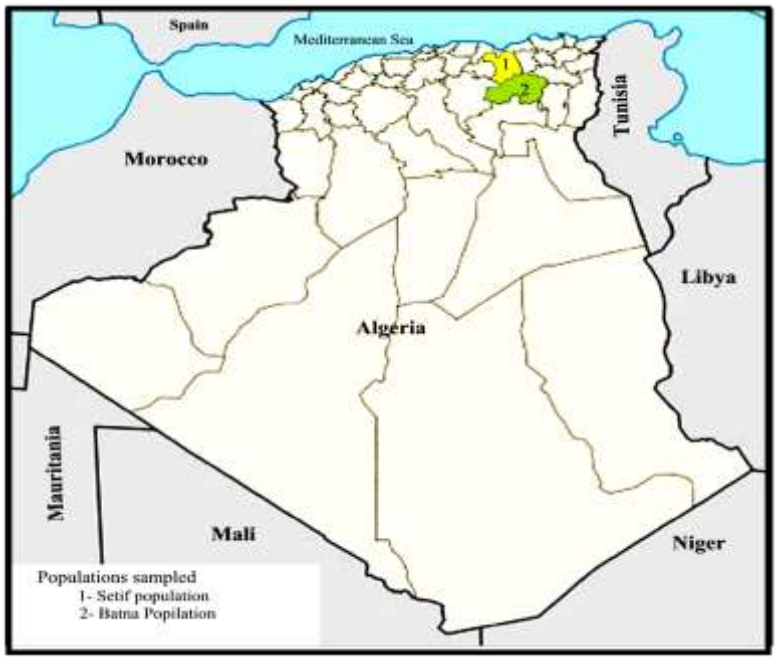

Figure 1. Populations of *H. cheirifolia* sampled

Antibacterial activity

The antimicrobial activities of *H. cheirifolia* essential, oil were evaluated against three Gram positive bacteria (*Staphylococcus aureus ATCC 25923, Enterobacter cloacae ATCC* 23355 and *Staphylococcus aureus* (MRSA) ATCC *29213)*, and seven Gram negative bacteria (*Serratia liquifaciens ATCC* 27592, *Salmonella typhimurium ATCC 13311, Serratia maecescens ATCC* 14756, *Escherichia coli ATCC 25922, Pseudomonas syringae ATCC* 53543, *Enterococcus fecalis* ATCC 49452 and *Shigella sp.* Bacterial inoculums were prepared from overnight broth culture in physiological saline (0.8 % of NaCl) to obtain an optical density ranging from 0.08 - 0.1 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 (6mm) were placed on inoculated agars, by test bacteria, filled with 10µl of undiluted and diluted essential oil (1/1, 1/2, 1/4 and 1/8 v/v of DMSO). DMSO was used as a negative control, and Gentamicin antibiotic as positive control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. All the tests were performed in triplicate, and the means were calculated as results. The Petri dishes

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DOI: 10.2478/asn-2020-0018

were incubated at 37°C for 18 to 24h aerobically. After incubation, inhibition zone diameters were measured and documented. The sensitivity to the essential oil was classified by the diameter of the inhibition halos as follows: not sensitive (–) for diameter less than 8 mm; sensitive (+) for diameter 9–14 mm; very sensitive (++) for diameter 15–19 mm and extremely sensitive (+++) for diameter larger than 20 mm [32].

Karyotypic analysis

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 (3:1v), 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 yellowish oils isolated by hydro distillation from the aerial part of *H. cheirifolia* were obtained in average yield of 1.50%. The obtained yield is high compared to the result obtained on Tunisian populations (0.01%) [7].

The essential oil was analyzed by means of (GC/MS). The chemical components of the essential oils identified for both populations are presented according to their appearance in the chromatograms (Table 1). Fifty seven compounds were identified in the essential oil of Batna population, which corresponds to 99.28% of the total oil. In the essential oil of Setif population, 55 components were identified, corresponding to a percentage of 98.57% of the total oil.

In Batna population the major compound is α-pinene with a high level (48.49%), spathulenol (3.3%), α-campholene aldehyde (3.1%) and 15 other compounds with percentages ranging from 1.0 to 2.8%. While Setif population is characterized by α-pinene (53.85%), germacrene-D (12.66%), caryophyllene oxid (2.6%), spathulenol (2.1%), germacrene-A (2.2%), β-selinene (2 %) and 8 other compounds with rates of 1 to 1.9%.

The chemical composition of Batna population differs from Setif population by the presence of germacrene-D with a significant rate at Setif and a low rate in Batna (12.66 and 2.64%) respectively. 42 compounds are present in the essential oil of both Populations (Batna and Setif). *H. cheirifolia* from Batna is characterized by the presence of 16 components, which are absent in Setif population and Setif population is characterized by the presence of 15 components which are absent in Batna population.

The distribution of chemical classes in the essential oils of *H. cheirifolia* is somewhat heterogeneous (Figure 2). The monoterpene hydrocarbons represent the highest rate with 53.6% for the population of Batna

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and 57.1% for that of Setif. Oxygenated monoterpenes are higher in Batna population oil with 18.9%, against 5.5% for the population of Setif. Hydrocarbon sesquiterpenes are represented by high oil content in Setif population (26.3%), whereas this chemical class represents only 13.5% in Batna population oil. While, oxygenated sesquiterpenes are present at a moderate level in the oils of both populations. The chemical results show that the composition of *H. cheirifolia* oils of the two populations is different. These two compositions probably correspond to two distinct chemical races. The oil of Batna population is characterized by the combination of α-pinene – spathulenol, while the oil of Setif population is characterized by α-pinene – germacene-D.

Table 1. Chemical composition of H. cheirifolia from Algeria

Localities		Batna	Setif	Localities		Batna	Setif
Yield %	kovats index KI	1.6	1.4	Yield %	KI	1.6	1.4
Total %		99.8	98.7	Total %		99.8	98.7
Tricyclene	917	0.1	0.1	Isogermacrene D	1425	0.0	0.2
α-thujene	922	0.2	0.1	Cis-eudesma-6,11-diene	1440	0.0	0.2
α-pinene	931	**48.5**	**53.9**	aromadendrene	1446	0.0	0.1
Camphene	945	0.3	0.1	α-humulene	1451	0.5	**1.1**
Thuja-2,4(10)-diene	949	0.0	0.2	Longifolene	1458	0.0	0.1
Sabinene	968	0.0	**1.3**	α-guaiene	1466	0.2	0.4
β-pinene	972	0.8	0.4	α-selinene	1469	0.2	0.5
Myrcene	985	0.0	0.4	β-copaene	1470	0.1	0.0
Menthatriene <1,7,4,8-ol>	999	0.4	0.0	**Germacrene-D**	1478	**2.6**	**12.7**
Δ3-Carene	1003	0.4	0.0	β-selinene	1483	0.8	**2.0**
α-terpinene	1012	0.4	0.1	bicyclo-germacrene	1491	0.3	**1.3**
Para-cymene	1019	**1.1**	0.2	noot katene	1503	0.0	0.2
Limonene	1024	0.6	0.2	Δ-cadinene	1513	0.5	0.4
γ-terpinene	1054	0.5	0.1	γ-cadinene	1519	0.0	0.1
Terpinolene	1080	0.3	0.0	Elemol	1543	0.4	0.2
Camphenone	1090	0.1	0.1	Epi-torilenol	1550	0.3	0.4
Linalool	1095	0.7	0.4	Germacrene-B	1555	0.2	0.0
α-campholene aldehyde	1121	**3.1**	0.7	1,5-epoxysalvial4(14)ene	1564	1.0	0.3
Pinocarveol trans	1136	**1.9**	0.7	Ionone dimethyl	1569	0.2	0.0
Camphor	1141	0.5	**1.5**	Spathulenol	1573	**3.3**	**2.1**
p-mentha-1,5-dien-8-ol	1146	**2.4**	0.0	Caryophyllene oxid	1578	**2.8**	**2.6**
Pinocarvone	1157	0.8	0.3	Salvial-4(14)-en-1-one	1588	0.0	0.6
Mentha-1,5-dien-8-ol-para	1166	0.0	0.3	Humulene epoxide 2	1605	**1.3**	**0.7**
Terpinene-4-ol	1175	**1.3**	0.3	α-farnesene (E, E)	1611	0.0	**1.2**
Acetophenone para methyl	1179	0.2	0.0	nor-copaanone	1618	1.0	0.4
Myrtenol	1189	**2.7**	0.6	γ-amorphene	1623	0.0	0.3
Verbenone	1200	**2.2**	0.4	Caryophyllene sis	1628	0.8	0.5
Trans carveol	1213	**1.4**	0.2	Cubenol	1639	0.4	0.4
Carvone	1238	0.4	0.0	Longifolene-D	1642	**2.4**	0.8
Methyl n nonyl ketone	1287	1.0	0.0	Caryphellenol-2	1665	**1.5**	**1.1**

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α-terpinyl isotanoate	1341	0.2	0.0	Jasmone-sis	1680	**1.7**	0.8
β-damascenone-E	1373	0.2	0.0	Tetradecanoic acid	1755	0.4	0.0
β-bourbonnene	1379	0.1	0.0	Selina-4,7-diene	1769	**1.0**	0.0
β-elemene	1384	0.5	0.0	2-Pentadecanone, 6,10,14-	1836	0.6	0.0
Germacrene-A	1384	0.0	**2.2**	Hexadecanoic acid	1956	**1.5**	0.2
β-caryophyllone 4,8 epoxy	1415	0.5	**1.9**	Abietadiene	2083	0.0	0.2
Chemical classes							
Monoterpenes hydrocarbons						53.6	57.1
Oxygenated monoterpenes						18.9	5.5
Sesquiterpenes hydrocarbons						13.5	26.3
Oxygenated sesquiterpenes						11.1	8.1
Other						2.7	1.7

The chemical composition of our essential oil extracted from *H. cheirifolia*, rich in α-pinene (51.2%), germacrene-D (7.7%), is different from that of the same species, which contains the (67.5%) [23]. The oil analysis of Oum Elbouaghi population (Algeria) by Segueni et al. [15] report that Valeranone, Monoethylhexyl phthalate and Drimenin are major compounds. While, on samples from the same region ar Boulechfar [25] reports α-pinene (49.9%) and 2-(1-Cyclopent-1-enyl-1-methylethyl) cyclopentanone (24.6%) as majority components.

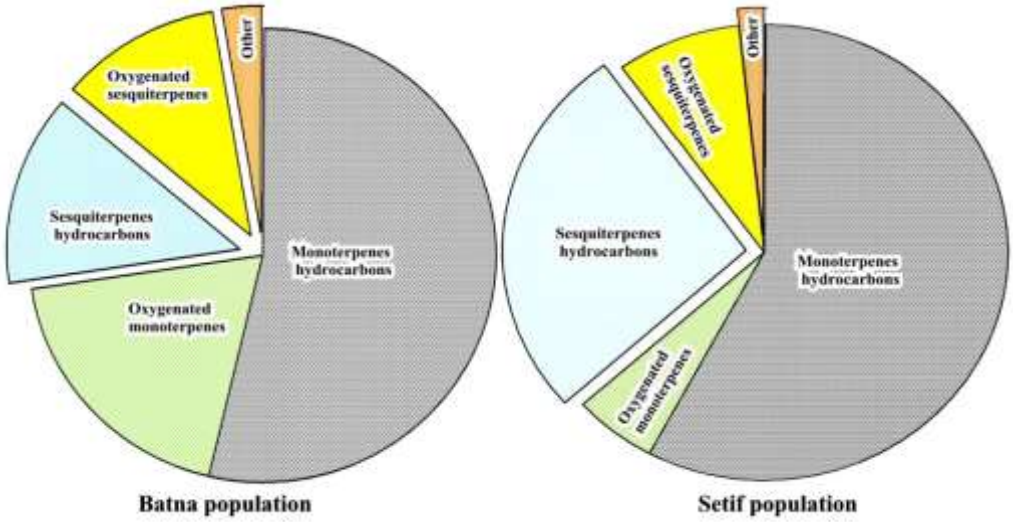

Figure 2. Chemical classes of *Hertia cheirifolia* essential oil

However, the main constituents of the essential oils of *H. cheirifolia* from Tunisia were α-pinene (70.4%) germacrene D (6.7%) and α-cadinol (3.2%) [20]. While the study of Attia et al. [21] on the same species of Tunisia, shows that thymol (61%), 2,6-dimethoxy-phenol (12.8%), Camphor (5.8 %) and Terpinene-4-ol (5.5%) are predominate. We notice significant differences between essential oils extracted from *H. cheirifolia*. The variation in the composition of essential oils among plant origin could be attributed to

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specific ecological factors [33-35]. Climatic parameters have a significant influence on the composition of essential oils of *H. cheirifolia* [14].

The comparison of the chemical composition of *H. cheirifoloia*, shows that the species of the genus *Hertia* are very close and differ only in one or two majority products. Samples of *H. angustifolia* from Iran, contain ß-pinene, ß-phellandrene, and α-pinene as major components [10-11]. The main constituents of the Essential oil of the aerial parts of *H. intermedia*, growing in Iran, were β-pinene, α-pinene, α-thujene, and β-phellandrene [11].

The antibacterial activity of essential oils of *H. cheirifolia* is evaluated by the disc method. The results are expressed by measuring the halos of inhibition diameter, after 24 hours of incubation at 37°C (Table 2).

Table 2. Inhibition zone diameter of *H. cheirifolia* essential oil (mm)

		Populations									
		Batna					Setif				
		Dilution				S*	*Dilution*				S*
Bacteria	G*	1/1	1/2	1/4	1/8		1/1	1/2	1/4	1/8	
Staphylococcus aureus ATCC 25923	25	10	7	8	6	+	13	7	10	0	+
Serratia liquifaciens ATCC 27592	10	15	9	8	0	+	10	7	0	0	+
Enterobacter cloacae ATCC 23355	0	10	8	8	7	+	0	0	0	0	–
Staphylococcus aureus (MRSA) ATCC *29213*	14	8	9	8	8	–	0	0	0	0	–
Salmonella typhimurium ATCC 13311	16	7	7	0	0	–	0	0	0	0	–
Serratia maecescens ATCC 14756	12	9	7	0	0	–	0	0	0	0	–
Escherichia coli ATCC 25922	14	0	0	0	0	–	0	0	0	0	–
Pseudomona syringae ATCC 53543	10	0	0	0	0	–	0	0	0	0	–
Enterococcus fecalis ATCC 49452	0	0	0	0	0	–	0	0	0	0	–
Shigella sp	15	8	9	7	0	–	0	0	0	0	–

* G = Gentamicin; *S = Sensivity (+) sensitive; (–) not sensitive.

This study investigated the in vitro antibacterial activity of the essential oils extracted from *H. cheirifolia*. The obtained results show a low antibacterial activity to absent against most of the tested bacteria. The only bacterial species with sensitivity to essential oils from both populations studded are *St. aureus* ATCC 25923 and *S. liquifaciens* ATCC 27592, while *E. cloacae* ATCC 23355 is sensitive to Batna population oils and resistant to Setif population oils (Figure 2).

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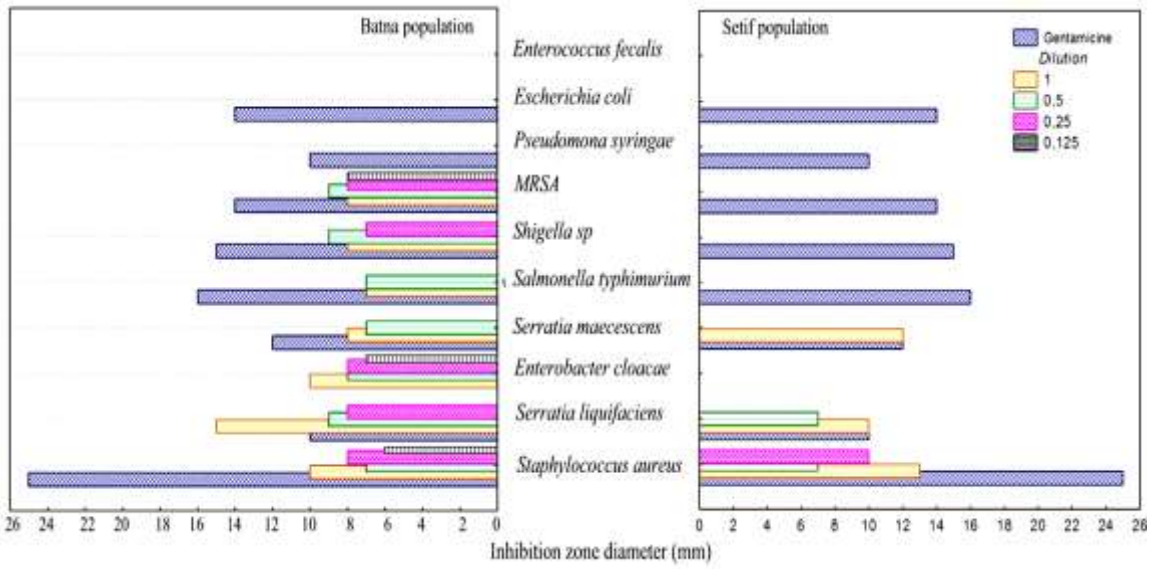

Figure 2. Antibacterial activity of *H. cheirifolia* essential oils

Authors have reported significant activity of *H. cheirifolia* essential oil on Gram-positive bacteria, particularly *S. aureus*. Gram-negative bacteria tested *(E. coli, P. aeruginsa and A. baumanii*) were less active [24]. Regarding our results, the moderate antibacterial activities of essential oils were found against the bacteria studded, while *Escherichia coli* ATCC 25922; *Pseudomonas syringae ATCC and Enterococcus fecalis ATCC 49452* are resistant to *H. cheirifolia* oils. In general, *H. cheirifolia* essential oil displayed varying degrees of antibacterial activity against the tested bacteria. The tested oil had moderate activity on *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* [25]. While Majouli and Kenani [36] found that, the use of the essential oil of *H. cheirifolia* could be considered as a natural source of inhibited growth of tested pathogenic bacteria. However, in a mixture of several molecules of essential oils, it is difficult to differentiate the component responsible for the antibacterial activity.

The extract of *H. cheirifolia* oil from Tunisia exhibited an antimicrobial property against bacterial strains [25]. The same antibacterial results were observed using the oils of the species of the genus *Hertia*. Bammou et al. [9] showed that essential oil of *H. maroccana* had a moderately activity against Gram negative and Gram positive. Akhgar et al. [11] showed that *H. intermedia* essential oil was restricted to positive gram bacterial growth and tested inactive on Gram-negative bacteria.

The observations of root cells meristematic at metaphase of *H. cheirifolia* from two populations (Batna and Setif) gave a diploid chromosome number 2n = 2x = 20, with a basic chromosome number (x= 10) (Figure 3).

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DOI: 10.2478/asn-2020-0018

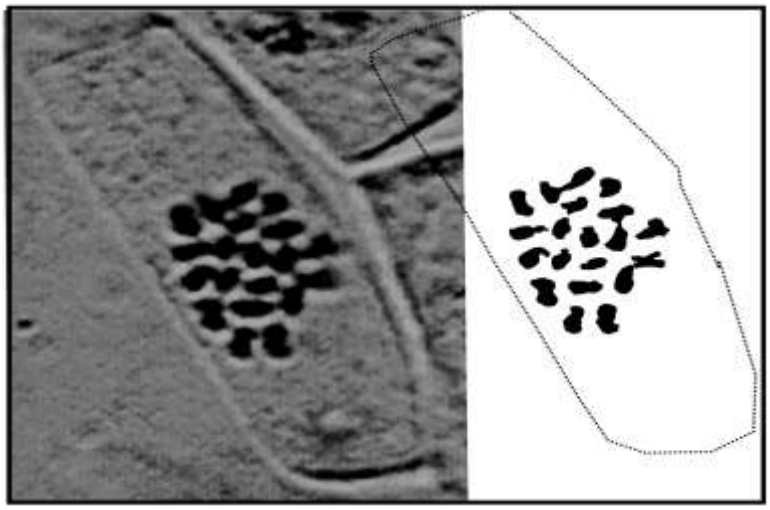

Figure 3. Karyotype of *H. cheirifolia* from Batna and Setif (2n = 2x = 20)

The karyological results are coherent with those obtained by the literature, whose chromosome number of *H. cheirifolia* is 2n = 2x = 20, with a basic chromosome number (x = 10) [28].

Conclusions

The phytochemical study of the species *Hertia cheirifolia* shows that it is rich in terpene compounds, with α-pinene and germacrene-d as major components. This species has variable phytochemical profiles that can be used for their individuality to several chemotypes. Two distinct chemotypes are highlighted, the α-pinene-Spathulenol chemotype of the Batna population, and the α-pinene-germacrene-D chemotype of the Setif population. The moderate antibacterial activities of essential oils were found against the bacteria tested. The chromosomal enumeration of root meristems showed a diploid chromosome number of 2n = 20.

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