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Essential Oils of *Hertia Cheirifolia* Leaves in Fructification Stage with High Anti Bacterial Activity

Bassa Nora[1,2*], Boulechfar Safia[1,3], Boutellaa Saber[1,4], Senoussi Mohamed Mourad[1,2] and Zellagui Amar[1,2]

1-Laboratory of Biomolecules and Plant Breeding, Nature and Life Sciences Department. Faculty of Exact Sciences and Nature and Life Sciences, University of Larbi Ben Mhidi, Oum El Bouaghi, Algeria.

2- Faculty of Exact Sciences and Nature and Life Sciences, University of Larbi Ben Mhidi, Oum El Bouaghi, Algeria.

3- Department of Biochemistry, Molecular and Cellular Biology, Faculty of Nature and Life Sciences, Mentouri University, 25000 Constantine, Algeria.

4- Life and Nature Science Department, Science and Technology Institute, University center of Mila, 43000, Algeria

**Corresponding author: norbassa@yahoo.fr*

ABSTRACT

The essential oil (EO) obtained by hydrodistillation of the *Hertia cheirifolia* leaves in fructification stage located in Oum El bouaghi (East of Algeria) have been studied by GC/MS. Fourty seven compounds were identified representing 95,21% of the essential oils. The main constituents were: α-Pinene (49.9 %), 2-(1-Cyclopent-1-enyl-1-methylethyl) cyclopentanone (24.6 %) and β-Phellandrene (2.1 %). In total, essential oil composition of *Hertia cheirifolia* leaves was considered as a rich source of hydrocarbon monoterpens. Moreover, the antimicrobial activity of the essential oil against four strains bacteria was studied. It was found that the most powerful effect was against *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC25923 with inhibition zone 27.66±0 and 26.33±0 at 2000 µg/mL successively.

Keywords :-Antibacterial activity; chemical composition; essential oil; GC/MS, *Hertia cheirifolia*; Oum El Bouaghi

Introduction

Microbial infections are caused by different microorganisms and are responsible for the most fatal diseases and the most widespread epidemics in the world. Anti-infective therapy is mainly based on the use of antibiotics which selectively inhibit certain metabolic pathways of microbes (Lewis, 2001; Hall & Mah, 2017).Over time, the number of bacteria resistant to antibiotics has increased and antibiotic resistance has become a global threat to the public health (Stefanović, 2018).Therefore, we find that research in this area is increasing significantly, especially the use of natural products such as essential oils. Essential oils are widely distributed in the plant kingdom, such as the asteraceae family, among its rare species *Hertia cherifolia*. *Hertia cheirifolia* (L) O.K also known as *Othonnopsis cheirifolia* Jaub. et Spach. is a small plant with yellow flowers, which grows in the border fields in the eastern part of Algeria and Tunisia (Pottier-Alapetite,1981; Beniston,1984). Previous phytochemical investigations on the genus *Hertia* report the presence of sesquiterpenes (Massiot et al.1990), Steroids extract (Ammar et al.2009) and essential oils (Zellagui et al. 2012, Segueni et al. 2017). Furthermore, very little study of the

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essential oils of this genus have been assayed for their antimicrobial activity against bacteria (Majouli et al.2016; Segueni et al. 2017). As a continuation of this study, here we report the results obtained of the analysis of the essential oil obtained from this species harvested at fructification stage of development in order to evaluate the *in vitro* their antibacterial activity.

Material and methods

Plant material

The leaves of *H.cherifolia* were collected during may 2017 (fructification stage) in Oum El bouaghi, Algeria(longitude: 7°06′48; latitude: 35°52′31; elevation: 925 m; annual precipitation: 412, 66 mm; semi-arid area). The plants were identified by Dr Zellagui Amar and a voucher specimen was deposited in the Laboratory of Biomolecules and Plant Breeding, University of Larbi Ben Mhidi Oum under number ZA 122(Fig1).

Figure1. Leaves and flowers of *Hertia cherifolia*

Extraction of the essential oil

Fresh leaves (100g) were hydrodistilled in a Clevenger-type apparatus for 3 h.

Gas chromatography - mass spectrometry

Analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions (Tab1): injector and transfer line temperatures 220 and 240°C, respectively; oven temperature programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1mL/min; injection 0.2 μL (10% n - hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of n -hydrocarbons, and by computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (Zellagui et al.2012).

Table 1: General information on GC-MS analysis performed

Column type	DB-5 capillary column (30m × 0.25 mm; coating thickness 0.25 μm)
Injection volume	0.2μL
Injector and transfer temperature	220°C and 240°C
Detector temperature	250°C

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Mode of injection	Split
Carrier gas	Helium

Antibacterial activity

The antibacterial activity was evaluated using standart bacterial strains *E. coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853 (G-), *Staphylococcus aureus* ATCC 25923 (G+) and another isolated strain *Klebsiella pneumonia.*

Table 2: Microbial strains used in this experiment

Strain	**Reference**
E. coli	ATCC 25922
P. aeruginosa	ATCC 27853
S. aureus	ATCC 25923
K. pneumonia	Isolated

Agar diffusion method

The method of diffusion on agar was chosen according to the guidelines of the National Committee for Clinical Laboratory Standards (NCLLS, 2002). Briefly, sterile Mueller-Hinton agar was used. Petri plates were inoculated with microorganism inoculation. The inoculum was prepared with a night culture of test microorganisms and the volume was adjusted to 0.5 McFarland standard turbidity. A sterile paper disc was prepared and fermented with 25, 100, 500 and 2000 µg/ml essential oil of *H. cheirifolia* and placed on agar plates containing one of the mentioned bacteria. The plates were left at room temperature for 30 min and incubated at 37 °C for 24 h. The antibacterial activity was determined by measuring the inhibition zone diameters of the essential oil of *H. cheirifolia*. All assays were performed in triplicate in three different experiments.

Results and discussion

Chemical composition of essential oil

The essential oil obtained by hydrodistillation of *H. cherifolia* leaves have been studied by GC/MS. Fourty seven compounds were identified representing 95,21% of the essential oils. The composition of volatile oil is given in table 3 and Figure 2. The main constituents were: α-Pinene (49.9 %), 2-(1-Cyclopent-1-enyl-1-methylethyl) cyclopentanone (24.6 %) and β-Phellandrene (2.1 %). *Hertia cheirifolia* leaves was considered as a rich source of hydrocarbon monoterpens**.** Comparing the results of the GC-MS analysis of this oil (in the same conditions) at this stage (fructification), showed a clear difference in the chemical composition with those extracted in the vegetative and flowering stages. The volatile fraction extracted in vegetative stage was dominated by (-)Drimenin (67.5%)(Zellagui et al.2012), While that harvested in flowering stage was dominated by phthalate, (33.71%), valeranone, (6.90%) and (-) Drimenin (6.71%) Segueni et al 2017. In addition Ounoughi et al. (2020) showed that the essential oils of aerial parts from H.cherifolia in flowering stage growing in setif region were dominated by Germacrene-D. Moreover, Rahali et al.2017 showed that the major constituents of the essential oil of leaves growing in Tunisia were α-pinene 35.63 %, This is consistent with our results in terms of the component, and the percentage is 49% in the leaves of

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the plant that grows in Algeria.
On other hand, several studies report the variability of a essential oils during the development cycle of the plant belongs to Asteraceae family

It is well known that genotypes, season of collection and geographical origin have a considerable effect on plant oils composition (Amirah et al.2012; Majouli et al.2016).On other hand, several studies report the varibility of a essential oils during the development cycle of the plant (Laxmi et al.1999; Farhadi et al.2020; Daghbouche et al.2020).

Table 3: Chemical composition of essential oil by GC-MS analysis of *H.cherifolia* leaves harvested at fruiting stage

pics	**Constituents**	**Retention time(Rt)**	**%**
1	α-pinéne	4.064	**49.9**
2	Camphene	4.339	0.2
3	β-Phellandrene	4.983	2.1
4	1,3,8-p-Menthatriene	6.636	0.9
5	Cyclobutane, 1,2-bis(1-methylethenyl)-,trans-	7.039	0.4
6	Thujone	11.376	0.1
7	Isothujol	13.141	0.3
8	4-Terpineol	14.620	0.1
9	Terpineol	15.192	0.1
10	Bicyclo[4.1.0]heptan-3-one, 7,7-dimethyl-4-methylene-,(1R)-	15.453	0.1
11	Carvacrol	19.192	0.8
12	α-Bourbonene	22.075	0.1
13	Cyclohexan, 1-ethenyl-2,4-bis(1-methylethenyl)-	22.339	0.9
14	Caryophylene	22.974	0. 7
15	1,4methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-Dimethyl-	23.566	0.1
16	α-Caryophyllene	23.853	0.5
17	α-Amorphene	24.317	0.2
18	α-Cubebene	24.679	1.3
19	α-Eudesmene	24.766	0.5
20	Eremophilene	24.961	0.4
21	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-(E,E)-	25.144	0.4
22	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-	25.251	0.2
23	ButylatedHydroxytoluene	25.403	0.1
24	Naphtalene, 1-2-3-4-4a-5-6-8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	25.685	0.6
25	Aromadendreneoxide-(2)	26.695	0.1
26	Spathulenol	26.886	0.6

Table 3. ***Cont.***

27	Davanone	27.006	0.3
28	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	27.191	0.8

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29	Cholestane, 4,5-epoxy-epoxy-,(4.α,5.α)-	27.387	0.2
30	1,3-Hexadiene,3-ethyl-2,5-dimethyl-	27.558	0.1
31	Varidiflorene	27.925	0.4
32	Guaoil	28.325	0.1
33	ë-Cadinol,	28.636	0.2
34	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	28.750	1.81
35	α-Gurjunene	29.274	0.4
36	2,3,4-trifluorobenzoic acid,tridec-2-ynyl-ester	30.024	0.2
37	Cyclohexan-1-ethanol, 1-hydroxymethyl-	30.217	0.1
38	2-(1-Cyclopent-1-enyl-1-methylethyl) cyclopentanone	31.504	**24.6**
39	Pregn-4-en-3-one, 20,21-[[(1,1-dimethylethyl) borylene]bis(oxy)-	31.820	0.7
40	Euparone	32.533	0.1
41	1,2-Benzendicarboxylic acid, bis(2-methylpropyl) ester	32.751	0.2
42	Acetic acid, 6-(1-hydroxymethyl-vinyl)-4,8a-dimethyl-3-oxo	34.473	0.2
43	Cyclohexane, 1,3,5-trimethyl-	35.228	0.1
44	Isomaltol	35.537	0.1
45	Procerin	35.863	1.6
46	Cycloisolongifolene, 8,9-dehydro-9-formyl	36.435	0.1
47	Jatamansone	38.834	1.2
	Total		**95,21%**

Monoterpens	Monoterpènes hydrocarbonés (%)	53,6 %	**55,1%**
	Monoterpènes oxygénés (%)	1,5 %	
Sesquiterpens	Sesquiterpènes hydrocarbonés (%)	7,4 %	**11,71**
	Sesquiterpènes oxygénés (%)	4,31 %	
Autres		28,4%	**28,4%**
Total (%)			**95,21%**

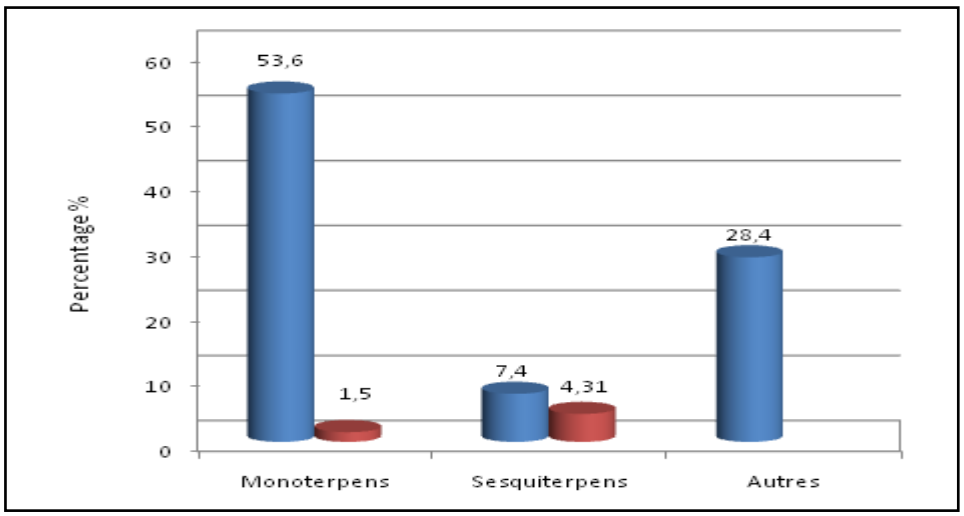

Figure2. Percentage composition of the subclasses in essential oils

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The *in vitro* antimicrobial activity of essential oil of *H.cherifolia* leaves harvested in fructification stage performed using the disc diffusion technique against four bacterial strains (Table 4). The results obtained are expressed as the mean zone of inhibitions of the three triplicates, which showed that essential oil extracted from *H.cherifolia leaves* prevented the growth of all the tested microorganisms with an inhibition zone medium diameter increasing proportionally with the concentrations of the tested samples. The obtained inhibition on bacteria strains varied from 7±0 to **27.66**±0 mm with a highest inhibition zone recorded with *Escherichia coli* ATCC25922 at 2000 µg/ml, and a considerable inhibition effect with the almost inhibition zone (**27.33**±0.30) at 500 µg/ml concentration. A high rate of inhibition was also recorded for *Staphylococcus aureus* ATCC25923 at 100, 500 and 2000 µg/ml concentrations. Overall, these results are very high compared to those obtained in the two stages (vegetative and flowering stage) with the same plant, part of plant (leaves) and the same geographical area. The most noticeable change, however, is the chemical composition, the presence of α-pinene with a high percentage 49.9.
Previous studies support our findings and claim that the effective antibacterial activity is attributed to the major phytochemical molecules, which are α-pinene (Rahali et al.2017). Additionally, similar to our results, extracted in various countries had substantial antibacterial action against many Gram-negative and Gram-positive bacteria, Majouli et al. (2016).showed that the essential oil of flowers exhibited a strong antibacterial activity against *Staphylococcus aureus.* Moreover, many references have proven the effectiveness of α-pinene in inhibiting and killing many gram-positive and gram-negative bacteria, also has been used for acting on antibacterial resistance modulation(Silva,2012; Salehi et al.2019; Joshiet al.2020)

Table 4. Antibacterial activity of *H.cherifolia*

Strains bacteria	25μg /1ml	100μg /1ml	500μg /1ml	2000μg /1ml
Escherichia coli ATCC25922	**20.33**±0.57	**26**±0	**27.33**±0.30	**27.66**±0
Staphylococcus aureus ATCC25923	**20.66**±0	**25**±0.15	**25.66**0.57	**26.33**±0.66
Pseudomonas aeruginosa ATCC27853	10.33±0.50	14.33±0.35	14.66±0	15.33±0.70
Klebsiella pneumonia	7±0	9.66±0.15	**23**±0.35	**25**±0

Conclusion

It could be concluded that *Hertia cherifolia* is rich in bioactive substances with high antibacterial capacity. Also, our study revealed the importance of choosing the plant part as well as the stage of growth, where we were able in the last, and according to the previous studies that we carried out in addition to this study, to confirm the best stage for harvested and thus obtaining the beneficial oil.

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Competing interests

Authors have declared that no competing interests exist.

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