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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.07.014

Essential oils from *Elaeoselinum asclepium*: Chemical composition, antimicrobial and antioxidant properties

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ARTICLE INFO

Article history:
Received 20 Jan 2016
Received in revised form 24 Feb, 2nd revised form 27 Apr, 3rd revised form 27 Jun 2016
Accepted 15 Jul 2016
Available online 26 Aug 2016

Keywords:
Elaeoselinum asclepium
Apiaceae
Essential oil
Antimicrobial activity
Antioxidant activity
Reducing power

ABSTRACT

Objective: To evaluate the chemical composition of the essential oil isolated from *Elaeoselinum asclepium* (L.) Bertol. (*E. asclepium*), and test the efficiency of the essential oil as an antimicrobial and antioxidant agent.

Methods: Essential oil was obtained from the aerial parts of *E. asclepium* by hydro distillation and analyzed by gas chromatography and gas chromatography coupled with mass spectrometry. We study for the first time the chemical composition of the essential oil of *E. asclepium*, followed by the *in vitro* antimicrobial activities, which were evaluated by agar diffusion method against six Gram-positive bacteria, five Gram-negative bacteria, and two fungi. In addition, The antioxidant activities were also investigated using assays of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity and ferric-reducing capacity.

Results: The analyzed essential oil of the aerial parts of *E. asclepium* was rich in α-pinene (43.9%), other compounds detected in appreciable amounts were sabinene (27.9%) and β-pinene (16.0%). The essential oil yields 1.2%, the IC_{50} values of essential oil in 1,1-diphenyl-2-picrylhydrazyl assay in the reducing power assay were 48.26 mg/mL and at 1 mg/mL, respectively. The absorbance value of essential oil at 700 nm was 0.956. The antimicrobial effect was higher on *Candida albicans* ATCC 1024 strain with the inhibition zone 14.5 mm than bacteria and molds.

Conclusions: The essential oil of *E. asclepium* has antimicrobial and antioxidant activities. These species may be used as an important source of natural antimicrobial and antioxidant agents.

1. Introduction

Aromatic plants, especially those from the Umbelliferae family are able to synthesize secondary metabolites, such as phenolic compounds, monoterpenes and sesquiterpenes [1]. Essential oil has been known to have antimicrobial and antioxidant proprieties [2–4]. It is worth mentioning that several studies have been conducted using different bacteria and fungi [5,6]. The activity of the chemical side was caused by the presence of terpenes and their oxygenated compounds, and each compound contributes to their biological activities [7–9].

The genus *Elaeoselinum* belongs to Umbelliferae family, Apioideae subfamily (Laserpitieae tribe) [10]. According to Algerian flora [11], the genus *Elaeoselinum* includes two species: *Elaeoselinum asclepium* subsp. *meoides* (Koch.) (*E. asclepium* subsp. *meoides*) (synonym: *Thapsia asclepium* L. or *Laserpitium asclepium* L.) Fiori, called locally Afs or Klikha, and *Elaeoselinum thapsioides* (Desf.) Maire (synonym: *Elaeoselinum fontanesii* Boiss.), called locally Becibsa.

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Foundation Project: Supported by Project Algero-Tunisienne with grant No. 280/11/07/2012.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

In Italy, *Elaeoselinum asclepium* (*E. asclepium*) has two subspecies [12]: *Elaeoselinum asclepium* subsp. *asclepium* (synonym: *Elaeoselinum hispanicum* (Lange) Pau), and *E. asclepium* subsp. *meoides* (Desf.) Fiori (synonym: *Elaeoselinum meoides* (Desf.) Koch ex DC).

In connection with the ongoing studies about secondary metabolites from Apiaceae, we are interested in the phytochemical investigation of the components of *E. asclepium* grown in Algeria. The aim of our work is to evaluate two biological activities of the aerial parts of these species: antibacterial and antioxidant activities. The antioxidant activity was evaluated using assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric-reducing capacity. On the other hand, the antimicrobial activity was evaluated against a wide range of different pathogenic microorganisms including bacteria, yeast and molds strains.

2. Materials and methods

2.1. Chemicals

DPPH, butylatedhydroxyanile (BHA) were purchased from Sigma–Aldrich (St Louis, MO, USA), potassium ferricyanide (LOBA chemicals), trichloroacetic acid (Scharlau chemicals, Espagne), dipotassium phosphate (ACROS, USA), Monopotassium phosphate (Panreac, Espagne) ferric chloride (Biomedicals) and other solvents, dimethylsulfoxide (DMSO) were used.

2.2. Plant material

The aerial part of *E. asclepium* (L.) Bertolis was collected in the region of Flifla (Skikda) on July 2013 during the period of blooming at 300 m above sea level. Then, they were freed from the impurities and dried in the shade at an ambient temperature. The collected plant was identified by Prof. Laouer Hocine, Laboratory of Natural and Biological Resources Valorization, Department of Biology and Plant Ecology. Voucher specimen was deposited in the herbarium of the same laboratory of University Ferhat Abbes, Setif 1 (Voucher number 15–2016).

2.3. Essential oil extraction

The dried aerial parts of the studied plant were subjected for 3 h to hydro distillation using a type of Clevenger apparatus. The obtained essential oil was stored at 4 °C until the time of test and analysis.

2.4. Essential oil analysis

2.4.1. Gas chromatography analysis

Chemical analysis of this essential oil was performed on Perkin–Elmer, Clarus 500 gas chromatograph, equipped with a flame ionization detector and HP 5MS 30 m × 0.25 mm × 0.25 μm film thickness capillary column. The detector, the injector and column temperatures were programmed at 300 °C, 230 °C and (60–280) °C, respectively, the initial rate of this latter equals 3 °C/min. Helium was employed as the carrier gas at rate of 1 mL/min.

2.4.2. GC–MS analysis

The GC–MS analyses were carried using a Hewlett Packard 5973–6890 GC–MS system operating on EI mode (fitted with a HP 5MS), using helium (1 mL/min) as the carrier gas. The first heating column was 60 °C as initial temperature and then it was increased gradually up to 280 °C with a 3 °C/min rate. The different compounds were identified by the comparison of their retention indices [13] obtained by using various *n*-alkanes (C_9–C_{24}). Also, the electron ionization-mass spectra of this analysis was compared with the NIST/NBS, Wiley libraries spectra and literature [14,15]. Furthermore, the marked phytochemicals was confirmed through comparing them with disposable authentic sample.

2.5. Antimicrobial assay

2.5.1. Microbial strains

Antibacterial activity of *E. asclepium* essential oil was tested against 11 strains of bacteria: *Staphylococcus aureus* ATCC 25923 (*S. aureus* ATCC 25923), *Micrococcus luteus* ATCC 4698 (*M. luteus*), *Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Klebsiella pneumonia* ATCC E47 (*K. pneumonia*), *Salmonella typhi* ATCC 19430 (*S. typhi*), *Salmonella enterica* ATCC 43972 (*S. enterica*), methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRSA), *Listeria inocula* CIP 74915 (*L. inocula*), *Bacillus cereus* ATCC 11778 (*B. cereus*) and *Enterococcus faecalis* ATCC 29212 (*E. faecalis*).

Antifungal activity was tested using *Aspergillus niger* 2CA936 (*A. niger*) and *Candida albicans* ATCC 1024 (*C. albicans*).

2.5.2. Agar diffusion method

The study of the antimicrobial activity was carried out following the method of Berghe and Vlietinck [16]. *E. asclepium* essential oil was dissolved at 100 mg/mL in 100% DMSO. Culture suspension (200 μL) of the tested microorganisms 10^6 CFU/mL of bacteria cells and 10^8 spores/mL of fungal strains were dispersed on Luria-Burtani and Sabouraud agar medium, respectively. After that, with a sterile borer, holes (6-mm diameter) were made and each hole was filled up with 60 μL of sample essential oil. The positive controls of bacteria and fungi were gentamycin and nystatine, respectively and DMSO as negative reference. The Petri dishes were placed in a cold room (4 °C) for 3 h, and then incubated for 24 h at 37 °C for bacteria, 48 h at 30 °C for *C. albicans* and 8 days for *A. niger* fungal strains. The evaluation of the antimicrobial activity was carried out by estimating the diameter of inhibition zones (mm). The work was repeated twice and the values are the average of two replicates.

2.6. Antioxidant assay

2.6.1. DPPH radical scavenging

The DPPH radical scavenging activity of *E. asclepium* essential oil was performed following the method of Kirby and Schmidt [17] with a few modifications. A volume of 500 μL of essential oil at various concentrations (1–30 mg/mL) was mixed with 375 μL of 99.8% ethanol and 125 μL of DPPH

solution (0.02%) as a free radical source. After that, the preparation was incubated for 1 h in the dark at room temperature. At the end, scavenging capacity was estimated spectrophotometrically by controlling the reduction in absorbance at 517 nm. In its radical form (purple color), DPPH has an absorption band at 517 nm which disappears upon reduction by an antiradical molecule (yellow color). A good radical scavenging activity has been interpreted by decreasing it in mixture absorbance. Synthetic antioxidant, BHA was used as positive reference. DPPH radical scavenging activity was calculated as:

$$\text{DPPH radical scavenging activity } \% = [(A_c - A_s)/A_c] \times 100$$

where, A_c is the absorbance of the control reaction, A_s is the absorbance of *E. asclepium* essential oil. Tests were performed in duplicate. IC_{50} values were estimated by a linear regression.

2.6.2. Ferric-reducing activity

The reducing power of *E. asclepium* essential oils was performed following the method of Yildirim *et al.* [18]. Sample solutions (0.5 mL) with various concentrations (0.5–10.0 mg/mL) of the essential oil added to 1.25 mL of 0.2 mol/L phosphate buffer (pH = 6.6) and 1.25 mL of (10 g/L) $C_6N_6FeK_3$ solution. Then, the preparation was incubated for half an hour at 50 °C. After this time, 1.25 mL of (100 g/L) trichloroacetic acid was added. A 1.25-mL aliquot of the supernatant from each sample mixture was added to 1.25 mL of distilled water and 0.25 mL of (1.0 g/L) $FeCl_3$ solution in a test tube. The absorbance was estimated at 700 nm after 10 min of incubation at room temperature against a blank.

There is a direct relationship between the concentration of essential oil, BHA and reducing power, where the increase of concentration of essential oil and BHA causes an increase in the reducing capacity. EC_{50} value (mg essential oil/mL) is the effective concentration at which the absorbance was 0.5 and it was obtained by interpolation from linear regression analysis [19].

3. Results

3.1. Essential oil analysis

The essential oil obtained from the aerial part of *E. asclepium* picked from the east of Algeria (Flifla-Skikda) was pale yellow with a pleasant and distinct odor for the flowering stage but colorless when the harvest is done in the last stage. The yield of this oil was 1.20% compared with the dry plant weight. Gas chromatography and gas chromatography–mass spectrometry (GC–MS) were used to analyze the essential oil produced. In Table 1, the different compounds of essential oil of *E. asclepium* (L.) Bertol. (quantity and quality) were found and arranged in order of elution in Rtx-1 column. Based on the results obtained, forty compounds are adopted which represent 99% of the full oil.

The highest percentages of compounds were monoterpenes such as α-pinene (43.9%), followed by sabinene (27.9%), β-pinene (16.0%), limonene (2.0%) and 4-terpineol (1.4%). However, there is a small amount of α-thujene (monoterpene bicyclique, 0.9%), myrtenal (0.8%), 3-carene (monoterpene bicyclique, 0.7%), myrcene (monoterpene-hydrocarbon, 0.7%), ρ-cymene (0.6%) and hydrocarbon $C_{10}H_{14}$ (0.6%).

Table 1
Chemical composition of the essential oil of *E. asclepium* analyzed by GC–MS.

Compounds	t_R (min)	RI^{Exp}	RI^{Lit}	%
Hydrocarbon C_8H_{16}	3.668	710	–	0.5
Dimethyl hexane C_8H_{18}	3.797	760	–	0.3
Cis-1,4-dimethylcyclohexane	3.864	780	782	0.1
Trans-1,3-dimethylcyclohexane	3.976	790	790	0.1
Cis-1,2-dimethylcyclohexane	4.520	842	828	Tr
α-Thujene	6.918	923	928	0.9
α-Pinene	7.260	933	936	43.9
Camphene	7.702	948	953	0.3
β-Pinene	8.565	971	978	16.0
Sabinene	8.785	976	976	27.9
Myrcene	9.105	986	991	0.7
α-Phyllandrene	9.731	1 003	1 005	0.1
3-Carene	9.839	1 006	1 010	0.7
α-Terpinene	10.146	1 013	1 018	0.2
ρ-Cymene	10.451	1 020	1 020	0.6
Limonene	10.718	1 028	1 031	2.0
(Z)-ocimene	11.310	1 040	1 040	Tr
(E)-ocimene	11.830	1 053	1 050	0.3
Cis-sabinenehydrate	12.291	1 065	1 068	Tr
Isoterpinolene	12.970	1 081	1 086	Tr
Trans-sabinenehydrate	13.601	1 097	1 096	Tr
Cis-pinenehydrate	14.640	1 120	1 122	Tr
α-Camphelenal	14.740	1 122	1 125	0.1
Hydrocarbon $C_{10}H_{14}$	15.356	1 136	–	0.6
Cis-sabinol	15.601	1 141	1 140	0.5
Trans-pinenehydrate	15.780	1 145	1 142	Tr
Pinocarvone	16.299	1 158	1 162	0.4
4-Terpineol	17.165	1 177	1 177	1.4
ρ-Cymen-8-ol	17.397	1 182	1 182	0.2
Myrtenal	17.783	1 191	1 193	0.8
Verbenone	18.281	1 202	1 204	Tr
Pulegol	18.842	1 214	1 213	Tr
ρ-Cymen-7-ol	22.044	1 287	1 287	0.3
Longicyclene or α-ylangene	25.640	1 371	1 372	Tr
Daucene	26.170	1 383	1 380	Tr
(E)-β-caryophyllene	27.441	1 413	1 418	Tr
(Z)-β-farnesene	28.900	1 449	1 443	Tr
γ-Himachalene	29.976	1 475	1 476	0.3
α-Selinene	30.049	1 488	1 494	0.4
Sesquiterpenoid	37.911	1 682	–	0.2

Tr: Traces.

3.2. Antimicrobial activity

The results indicated that the essential oil of *E. asclepium* was active against the microorganisms assayed (Table 2). The essential oil tested showed various degrees of antibacterial and antifungal activities against most of bacteria and fungi tested, it was active against *M. luteus* ATCC 4698 (10.0 mm) and *B. cereus* ATCC 11778 (10.0 mm), however, no activity was observed on *S. typhi* ATCC 19430, *E. faecalis* ATCC 29212 and *L. inocula* CIP 74915 bacteria. The other bacterial strains (*S. aureus* ATCC 25923, *S. enterica*, *P. aeruginosa*, *K. pneumonia* ATCC E47) have a diameter of inhibition zones ranging from 8.0 to 9.0 mm. *C. albicans* was the most susceptible with an important inhibition zone of 14.5 mm. At this concentration, inhibition diameters shown by the essential oil were lower than those induced by gentamicin and nystatin.

Table 2
Inhibition diameters in mm of *E. asclepium* essential oil (mm).

Bacterial strains	Essential oil	Standard	DMSO
S. typhi ATCC 19430	NI	35.0 ± 1.0[a]	–
E. coli ATCC 25922	12.0	29.0 ± 1.0[a]	–
M. luteus ATCC 4698	10.0	24.0 ± 1.0[a]	–
S. enterica ATCC 43972	8.0	13.0 ± 2.0[a]	–
S. aureus ATCC 25923	8.0	25.0 ± 1.0[a]	–
E. faecalis ATCC 29212	NI	30.0 ± 2.0[a]	–
MRSA	8.0	27.0 ± 1.0[a]	–
B. cereus ATCC 11778	10.0	30.0 ± 2.0[a]	–
L. inocula CIP 74915	NI	26.0 ± 1.0[a]	–
P. aeruginosa ATCC 27853	8.0	35.0 ± 2.0[a]	–
K. pneumonia ATCC E47	9.0	16.0 ± 1.0[a]	–
C. albicans ATCC 1024	14.5	21.0 ± 0.1[b]	–
A. niger 2CA936	NI	11.0 ± 0.1[b]	–

NI: No inhibition zone; [a]: Gentamicin; [b]: Nystatin.

3.3. Antioxidant activity

3.3.1. DPPH test

The examined essential oil could reduce the stable radical DPPH into DPPH-H. The IC_{50} value of the essential oil of *E. asclepium* was 48.26 mg/mL, whereas the IC_{50} of BHA was 12.80 μg/mL. This activity was concentration-dependent.

3.3.2. Ferric-reducing activity

The EC_{50} value of the essential oil of *E. asclepium* was 0.513 mg/mL, whereas the EC_{50} of BHA was 22.24 μg/mL. The essential oil exhibited low reducing potential in comparison with BHA. There is a direct relationship between the concentration of essential oil, BHA and reducing power, where the increase of concentration of essential oil and BHA causes an increase in the reducing capacity.

4. Discussion

4.1. Chemical analysis

The essential oil's yield obtained in this study (1.2%) was relatively higher compared to the species yields in the same family: *Daucus carota* ssp. *carota* fruits ranged from 0.8% to 1.6% (v/w), roots up to 0.2% and leaves 0.3% from Ober-Sankt-Veit (v/w) [20], *Astrodaucus persicus* aerial parts 0.6%–0.9% (v/w) while the roots 0.1% (v/w) [21], *Anthemis pedunculata* 0.10% (w/w) and *Anthemis punctata* 0.26% (w/w) [22], *Daucus gracilis* 0.68% (v/w) [23]. However, the essential oil of *Astrodaucus persicus* roots gave a very low rate which was 0.1% (v/w) [21]. Our result is in accordance with other analyses showing similar or low quantities of essential oils: *Foeniculum vulgare* fruit ranged from 1.1% to 2.9% (v/w) [24], *Distichoselinum tenuifolium* flowering umbels yielded 0.1% (v/w), ripe umbels 2.0%–2.6% (v/w) [25]. According to Fellah *et al.* [26], variations in yields could be attributed to several factors such as the extraction technique and the collection period of the plant material [23]. In the study of Guido Flamini *et al.* [27], the yields of *Daucus sahariensis* Eos were different and this is based on the plant's growth phase. In fact, we have observed a lower yield of 0.27% for plants collected at the flower-budding phase in comparison to the higher yields of 0.63% and 0.68% obtained for plants harvested at the flowering and fruiting phase, respectively. Bader *et al.* [10] reported that the highest essential oil of *E. asclepium* subsp. *meoides* growing in Sicily yield was obtained from the ripe fruits (3.8%), followed by the roots (2.2%) and the aerial parts (0.95%). Zheljazkov *et al.* [28] added the effect of the extraction time.

The results of essential oil's chemical composition are far from those of Evergetis *et al.* [29], which showed that the essential oil of *E. asclepium* is formed by: α-pinene (27.41%), β-pinene (5.23%), myrcene (5.98%) and β-phellandrene (1.63%) are similar for the essential oil of *Elaeoselinum gummiferum* plant [30]. In addition, sabinene, myrcene, α-pinene, β-pinene and camphene were evaluated as main constituents, according to essential oil content of nine commercial varieties of *Daucus carota* ssp. *sativus* (fruits/seeds) and *Daucus carota* ssp. *major* (flowers and fruits) [31]. On the other hand, the chemical analysis of the essential oil of *Elaeoselinum* W.D.J. Koch genus has initially shown the presence of trans-β-farnesene, α-phellandrene, α-copaene, germacrene-D, bicyclogermacrene and α-humulene [29].

Either α-pinene or α + β-pinene as the main constituent it is a common character for all the species, with the exception of *Elaeoselinum tenuifolium* fruits (where myrcene (47.9%) and sabinene (24.3%) were the main components), and one sample of *Elaeoselinum fontanesii* fruits, in which limonene (32.9%) was the principal compound. The analysis of the essential oil of *E. asclepium* subsp. *meoides* revealed the presence of kaurane and epi-13-manoyl oxide in very small amounts. The presence of manoyl oxide in *Elaeoselinum gummiferum* [30] confirms the ability of this genus to synthesize labdanediterpenes [10]. *Distichoselinum tenuifolium*, distributed widely on the province of Algarve, South Portugal, (=*Elaeoselinum tenuifolium*) is the unique species of the genus. High percentage of myrcene (48%–85%) was not detected in the essential oil of *Elaeoselinum* species [25,30,32].

The study of the larvicidal activity of essential oil of *Oenanthe pimpinelloides* L. (Apiaceae), which essentially contains monoxygenated monoterpenes, indicated that this oil has a high activity against *Culex pipiens* larvae with IC_{50} = 40.26 mg/L compared to the essential oil of *E. asclepium* (L.) Bertol, which contains pinenes and oxygenated monoterpenes, the activity of this oil was less with IC_{50} = 96.96 mg/L. The results of this study suggest that the monoxygenated monoterpenes own strong insecticidal activities against *Culex pipiens* L. [29]. According to Bakkali *et al.* [33], the extraction of essential oil under the same conditions from the same part of the plant which should be planted in the same soil, in the same climate and the same season of harvest gives an essential oil with constant composition.

4.2. Microbial test

The propagation of drug resistant pathogens is considered as one of the most dangerous threats to effective treatment of microbial infections. Over the ages, essential oils and other extracts of plants have shown their effectiveness as sources of natural products and mainly for their potential utilization as other remedies for numerous contagious diseases too [34].

Both *E. asclepium* and *Daucus setifolius* essential oils showed low antibacterial activity with all strains tested with varying zone of inhibition (from 6 mm to 13 mm) [35]. The antibacterial activity of *Daucus carota* (*D. carota*) root oils may be slightly related with their main constituents, such as (Z)-a-santalol (14.1%), caryophyllene oxide (10.6%), and

spathulenol (9.8%) [36]. Whereas, the sensitive of the essential oil of *Daucus setifolius* Desf, Bejaia population, was dependent on β-pinene content (41.1%) [35]. *P. aeruginosa* showed natural resistance to many antibiotics [37,38]. On the other side, Satrani *et al.* [39] reported that *E. coli* was more sensitive than *S. aureus* to the essential oils from *Ammi visnaga* (L.) Lam. (Maroc), this antibacterial activity was due to their major components: linalol (22.71%) and methyl-2-butyrate d'isoamyle (27.68%). *C. albicans* was also inhibited by the essential oil of *Daucus gracilis* and the susceptibility was probably due to the high content of the elemicin (35.3%) and the geranyl-acetate (26.8%) in the essential oil [23]. Mileski *et al.* [5] have established that the fractions containing the high concentration of α-pinene and sabinene that inhibited effectively the growth of microorganisms; especially against yeast *C. albicans* which confirm our results with the same strain.

Our work results were in the same axis with several studies which have shown that the essential oils are slightly less active against Gram-negative than Gram-positive bacteria [40,41].

The antimicrobial agents such as essential oil and the majority of antibiotics can easily enter inside Gram-positive bacteria because the cell wall of this type of bacteria is rich on mucopolysaccharides and proteins but less on phospholipids [41,42].

Hajji *et al.* [40] showed that the presence of oxygenated monoterpenes, monoterpene hydrocarbons and aldehydes in essential oils have the ability to inhibit the process of breathing and ions circulation and consequently the destruction of the bacterial cell. The biological activity of essential oil is often attributed to their major components, the moderate antibacterial activity of *E. asclepium* essential oil can be accorded to high amount of monoterpenes such as α-pinene (43.9%) and possibly because of the interaction effect between the different elements of oil [8,25,43].

So in our opinion, the essential oil's antimicrobial activity is thanks to the interactive effect between the major and minor compounds of this essential oil. Based on these results, it can be said that the essential oil of *E. asclepium* is able to inhibit the growth of yeast (*C. albicans*) and both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*, *M. luteus*, *B. cereus*) bacteria. The used concentration of DMSO does not have any antimicrobial effect.

4.3. Antioxidative assay

The DPPH scavenging activity of *E. asclepium* essential oil (IC_{50} = 48.26 mg/mL) was weaker than BHA (IC_{50} = 12.80 μg/mL). However, the IC_{50} of Algerian *D. carota* L. essential oil was 40.97 mg/mL [36] which is similar to the IC_{50} of our essential oil. The works of Meliani *et al.* [36] have shown that the essential oils of Algerian *D. carota* L (stems/leaves) consist primarily of monoterpenes such as α-pinene, sabinene, β-pinene, limonene, myrcene, terpinene-4-ol. It is observed that the chemical composition of *E. asclepium*'s essential oil (Table 1) is relative to *D. carota* L. which reflects the similar DPPH scavenging activity.

The antioxidant activity relies on the chemical composition. It seems that the essential oils which contain oxygenated sesquiterpenes, monoterpenes and phenolic compounds have greater antioxidant properties [9,36,44].

The essential oil's compounds have a direct association with the antioxidant activities. This may be the result of the presence of high ratio of the main constituents and also the result of the presence of other compounds in low concentration or the synergy between them.

4.4. Ferric-reducing activity

In general, the ferric-reducing test is mainly employed to see the capacity of natural antioxidant in giving an electron or hydrogen [45]. According to various works, there is a direct relation between antioxidant ability and ferric-reducing of some bioactive molecules. For that reason, it is amply believed that the highest absorbance at 700 nm indicates a great reducing power [40]. The reducing power of BHA was significantly more evident relating to the essential oils of aromatic plants.

High percentage of essential oil increased the antioxidant capacity, it means that there are certain elements in essential oil of *E. asclepium* which are electron donors and might react with free radicals to stabilize them and to end radical chain reactions [46]. It can be concluded that this impact could be related to the presence of certain contents that have antioxidant capacity, and also to possible antagonistic and synergistic impact of contents and efficient groups in the essential oil [47].

It may be suggested that like all the medicinal plants, *E. asclepium* contains active substances (α-pinene and β-pinene) and other bioactive substances potentially useful for medicinal properties and as natural food preservation. In the present study, it was found that the essential oil of the aerial part of *E. asclepium* possesses antimicrobial, antioxidant and radical scavenging activities.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to Professor Moncef Nasri, Director of Laboratory of Enzyme Engineering and Microbiology, National School of Engineers of Sfax, Tunisia for his great contribution to this work. The authors would like to thank Professor Serkos A. Haroutounian of the Chemistry Laboratory, Agricultural University of Athens, Greece for the GC–MS analysis. This work was financially supported by Project Algero-Tunisienne with grant No. 280/11/07/2012.

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