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Effects of different ecological conditions and extraction techniques on the quality of volatile oils from flaxleaf fleabane (*Erigeron bonariensis* L.)

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The objective of this study was mainly to compare the chemical composition of essential oils from the whole parts of *Erigeron bonariensis* obtained by traditional hydrodistillation technique and by supercritical fluid extraction recognized as an alternative efficient method. A comparison of the effects of different ecological conditions on the chemical composition of essential oil samples collected in different localities, Monastir (Tunisia) and Cagliari (Sardinia, Italy), has been also performed. Thus, interestingly gas chromatography/mass spectrometry (GC/MS) analysis showed that the volatile fraction prepared by supercritical fluid extraction from Tunisian *E. bonariensis* was richer in terpenes components (90.6%) compared with the essential oil obtained by traditional hydrodistillation (86.2%). Chemical analysis revealed that although roughly the same compounds were extracted using both supercritical fluid extraction (SFE) and hydrodistillation (HD) but with different percentages. Furthermore, the essential oil of Tunisian *E. bonariensis* obtained by traditional hydrodistillation has high content of caryophyllene oxide (18.7%), spathulenol (18.6%) and *α*-curcumene (10.2%) whereas Sardinian essential oil was richer in *Cis*-lanchnophyllum ester (14.2%) and (*E*)-*β*-farnesene (12.0%).

Key words: *Erigeron bonariensis* L., essential oils, supercritical carbon dioxide extraction, chemical composition, gas chromatography/mass spectrometry (GC/MS).

INTRODUCTION

Essential oils deriving from medicinal plants are considered of paramount importance in human life since they are widely used in pharmaceutical, agronomic, food, cosmetic, perfume industries and as natural remedies (Bakkali et al., 2008). Plants of the genus *Erigeron* involves about 150 species occurring in the northern Hemisphere zone, mainly in North America (Nazaruk et al., 2009), they have a wide range of applications in folk medicine to treat a number of infirmities. In Asian traditional medicine, flowers of *Erigeron bonariensis* are used topically to treat gastrointestinal problems. The leaves have been reported as laxative. Roots of *Erigeron*

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species have been reportedly used in diarrhea and dysentery treatment (Maia et al., 2002; Bukhari et al., 2013).

The flaxleaf fleabane, *E. bonariensis* belongs to the Asteraceae family (Dobignard and Chatelain, 2011). It is a spontaneous annual or short-lived perennial weed growing in the Tunisian territory with an erect stem branched above and lateral branches overtopping the main axis with Inflorescence, usually terminal cluster, consisting of a large number of greenish, pinkish, white or cream very small flower heads (4 to 12 mm). Fruits: very small akenes in size topped with a pappus (type "Dandelion"). Flowering period extends from May to December (Jauzein, 1995).

Chemical composition of essential oils extracted by traditionally hydrodistillation (HD) technique from leaves, stems, flower heads and roots of *E. bonariensis* L. collected at different stages (spring, summer and autumn) have been previously investigated (Mabrouk et al., 2011). Summer samples were rich in Matricaria ester (1.6 to 76.4%) and caryophyllene oxide (1.6 to 22.6%). According to the literature, ecological factors of the biotope can affect growth as well as the quality and quantity of generated metabolites and the extraction techniques can affect the quality of the essential oil and its medicinal properties. Essential oils are mainly produced via distillation processes like HD and steam distillation (SD), both processes are less expensive but they can induce thermal degradation, hydrolysis and water solubilization of natural fragrances. These disadvantages can be avoided by using CO_2 supercritical fluid extraction (SFE) as an innovative method allowing to improve the quality of product extraction (Bukhari et al., 2013). Oils obtained by supercritical fluid extraction from aromatic plants are actually designated as volatile oils differentiating from the essential oils (Coelho et al., 2012). The main objective of this work is to study the effects of variability of the ecological factors and of extraction techniques on the quality of volatile oils from flaxleaf fleabane *E. bonariensis*.

MATERIALS AND METHODS

Plant

Aerial parts from *E. bonariensis* L. were collected in July, 2011 from two different localities: from roadsides and more or less anthropised wastelands within the Monastir (Tunisia) region and from wastelands within Cagliari (Sardinia, Italy). Voucher specimens have been deposited at Chemistry Department, Faculty of Sciences of Monastir, Tunisia and University of Cagliari, Italy.

Hydrodistillation

Hydrodistillation was performed for 4 h in circularly clevenger-type apparatus up to exhaustion of the oil contained in the matrix (Sapra et al., 2010). About 100 g of dried plant material was charged. Essential oils from Tunisian and Italian *Erigeron* were obtained with yields of 0.19 and 0.15%, respectively.

SFE apparatus

Supercritical CO_2 (purity 99%) extraction of volatile oil from Tunisian *E. bonariensis* was performed in a laboratory apparatus (Figure 1) equipped with a 400 cm^3 extraction vessel, E, operated in the single-pass mode by passing CO_2 through the fixed bed of charged vegetable particles (150 g of *Erigeron* grounded material). Waxes and essential oil were recovered in two separator vessels connected in series, S_1 and S_2 (Figure 1), 300 and 200 cm^3, respectively (Marongiu et al., 2004). The cooling of the first separator was achieved by using a thermostated bath (-10°C), accuracy of 0.1°C). The second separator allowed the discharge of the liquid product at desired time intervals. A high pressure diaphragm pump, P, with a maximum capacity of 6 kg/h, pumped liquid CO_2 at the desirable flow rate (1 kg/h). Carbon dioxide was then heated to the desired extraction temperature in a thermostated oven (40°C) (accuracy of 0.02°C). The extraction was carried out in a semibatch mode by charging the vegetable matter in the vessel, followed by a continuous flow solvent. Carbon dioxide flow was monitored by a calibrated rotameter located after the last separator. The CO_2 delivered during an extraction test was measured by a dry test meter. Temperatures and pressures along the extraction apparatus were measured by thermocouple and Bourdon-tube test gauges, respectively. Pressure was regulated by high pressure valves under manual control. Experiments were carried out at 90 bar and 40°C in the extraction section. In the first separator the temperature was set at -10°C and the pressure at the same value as the extraction section. The second separator was set at 15 bar and 10°C. Extractions were carried out in a semi batch mode: batch charging of vegetable matter and continuous flow solvent. About 150 g of material were charged in each run. The SFE volatile fraction from *E. bonariensis* collected from Monastir, Tunisia was obtained with a yield of $1.9.10^{-2}$%.

GC/MS analysis

GC/MS analysis was carried out using a gas chromatograph (Agilent, Model 6890N, Palo Alto, CA, USA) equipped with a split-splitless injector, an Agilent model 7683 autosampler and an Agilent HP5-MS fused silica column (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 µm). The GC conditions included programmed heating from 60 to 246°C at 3°C min^{-1}, followed by 20 min under isothermal conditions. The injector was maintained at 250°C. Helium was the carrier gas, at 1.0 ml min^{-1}. Samples were diluted in hexane with a dilution ratio of 1:100 and (1 µl) was injected in the split mode (1:20). The GC was fitted with a quadrupole mass spectrometer with an Agilent model 5973 detector. The MS conditions were as follows: ionization energy, 70 eV; electronic impact ion source temperature, 200°C; quadrupole temperature, 150°C; scan rate, 3.2 scan s^{-1}; mass range, 30 ÷ 480 u. The software Agilent MSD ChemStation E.01.00.237 was used to analyse the mass spectra and chromatograms (Hammami et al., 2013). The linear retention indices (RIs) for all of the compounds were determined by injection of a hexane solution containing the homologous series of C_8-C_{26} n-alkanes (Van Den et al., 1963). The identification of the essential oil constituents was accomplished by comparison of their retention indices and their mass spectra with the literature data and the mass spectra databases, including HPCH2205 (Adams, 2007) and W8N05ST (Wiley ver. 8.0 & NIST,

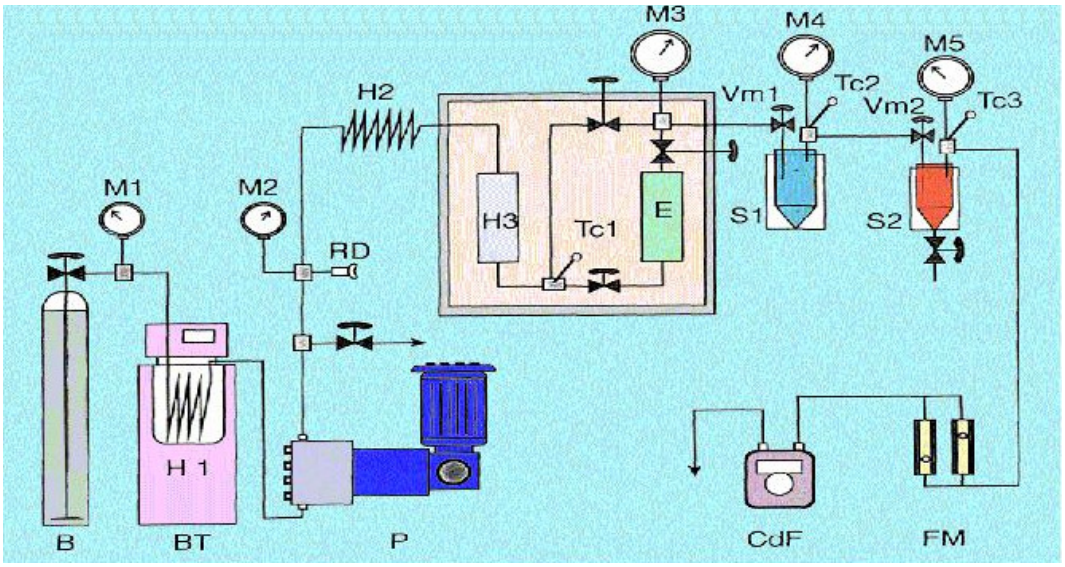

Figure 1. Scheme of the supercritical fluid extraction apparatus.

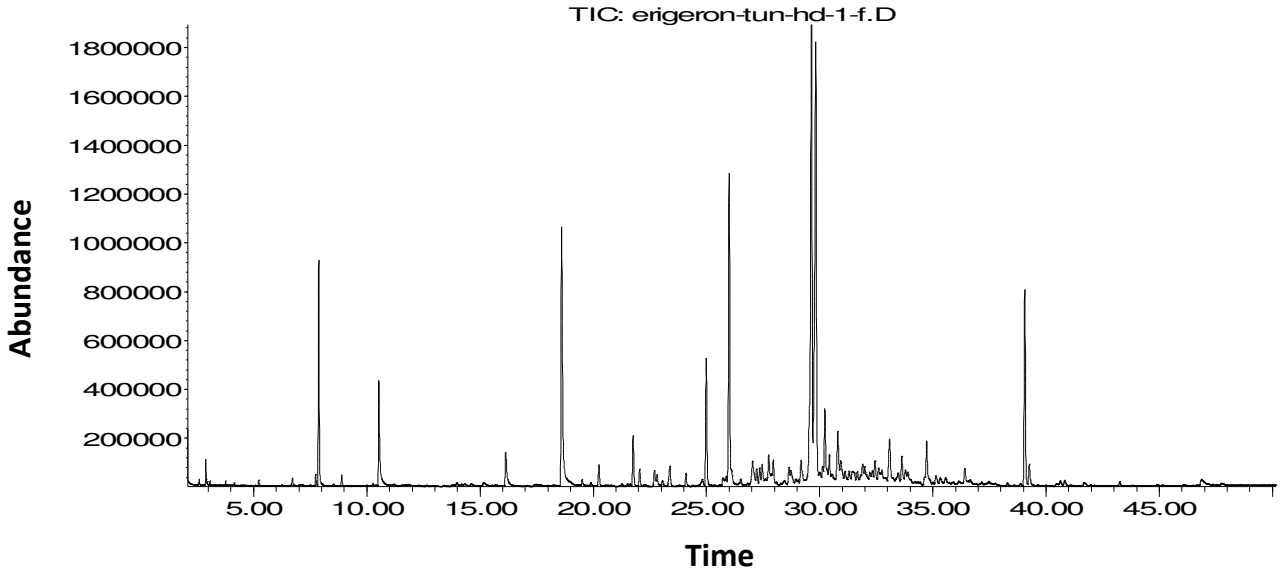

Figure 2. The GC/MS chromatogram of *E. bonariensis* aerial parts collected from Monastir, Tunisia (essential oil prepared by HD).

ver. 5.0). The chromatographic results were expressed as area percentages (GC) calculated without any response factor (Table 1).

RESULTS

E. bonariensis specimens were identified by Dr. Ridha El Mokni, Laboratory of Botany and Plant Ecology, Faculty of Sciences of Bizerta, Jarzouna, Bizerta, Tunisia. Within the aim to compare the effects of extraction techniques on the essential oil qualities, the chemical compositions of HD essential oils and SFE volatiles extracted from aerial parts of *E. bonariensis* L. collected from Monastir (Tunisia) have been determined via GC/MS analysis (Figures 2 and 3). Qualitative and quantitative compositions are presented in Table 1. Thus 30 components have been identified, we noticed that the number of the

Table 1. composition of *E. bonariensis* aerial parts oil isolated by CO_2 extraction (SFE) and by hydrodistillation (HD), respectively; the percentages are based on GC peak areas (sample harvested from Tunisia).

Retention time (min)	Retention idex (I_R)	SFE (% Area)	HD (% Area)	Compound	Identification
2.86	800	-	0.2	n-octane	MS, RI
7.88	1026	0.8	5.1	limonene	MS, RI
8.53	1050	-	-	(*E*)-*β*-ocimene	MS, RI
10.53	1103	-	3.5	n-nonal	MS, RI
11.54	1132	-	-	(*E,E*)-2,6-dimethyl-1,3,5,7-octatetraene	MS, RI
16.13	1241	-	1.4	carvone	MS, RI
18.60	1300	12.9	9.8	carvacrol	MS, RI
20.25	1340	-	0.7	silphinene	MS, RI
21.76	1376	1.4	1.6	modheph-2-ene	MS, RI
22.05	1383	-	0.5	α-isocomene	MS, RI
23.39	1416	-	0.9	(*E*)-caryophyllene	MS, RI
24.77	1453	-	-	*α*-humulene	MS, RI
24.99	1456	7.3	4.0	(*E*)-*β*-farnesene	MS, RI
25.88	1480	-	-	γ-muurolene	MS, RI
26.00	1481	6.8	10.2	α-curcumene	MS, RI
26.50	1494	-	-	bicyclogermacrene	MS, RI
27.04	1507	-	1.4	α-bulnesene	MS, RI
27.36	1516	0.7	1.0	cis-lachnophyllum ester	MS, RI
27.75	1526	-	1.0	matricaria ester	MS, RI
29.17	1563	-	1.0	(E)-nerolidol	MS, RI
29.64	1575	7.2	18.6	spathulenol	MS, RI
29.82	1579	1.0	18.7	caryophyllene oxide	MS, RI
30.23	1590	-	1.9	salvial-4(14)-en-1-one	MS, RI
30.43	1595	-	0.5	syn-anti-anti-helifolen-12-al A	MS, RI
30.80	1605	-	2.0	humulene epoxide II	MS, RI
31.80	1634		-	caryophylla-4(12),8(13)-dien-5α-ol	MS, RI
31.90	1635	-	0.3	isospathulenol	MS, RI
32.44	1650	-	0.7	α-cadinol	MS, RI
33.63	1682	-	0.8	germacra-4(15),5,10(14)-trien-1-α-ol	MS, RI
39.06	1838	53.2	6.1	neophytadiene	MS, RI
Total identified		91.3	91.9	-	
Terpenes		90.6	86.2	-	
Monoterpenes		13.7	16.3	-	
Diterpenes		53.2	6.1	-	
Sesquiterpenes		23.7	63.8	-	

NIST/EPA/NIH. Mass spectral library. Gaithersburg: National Institute of Standard and Technology.

volatiles components extracted by CO_2 supercritical fluid method (9 components) is lower than those detected via traditionally hydroditillation (24 constituents). The volatiles in the SFE oil were richer in neophytadiene (53.2%) and carvacrol (12.9%) than those produced by hydrodistillation, whereas caryophyllene oxide (18.7%), spathulenol (18.6%) and *α*-curcumene (10.2%) were present in higher percentage in the essential oil obtained via hydrodistillation comparing with the SFE volatiles (1.0, 7.2 and 6.8%, respectively).

On the other hand and within the objective to study the effects of environmental conditions such as the soil type, available water and the temperature on the composition and content of essential oil, HD essential oils of *E. bonariensis* growing in Tunisia and in Italy were analyzed via GC/MS (Figures 2 and 4). The HD Tunisian essential

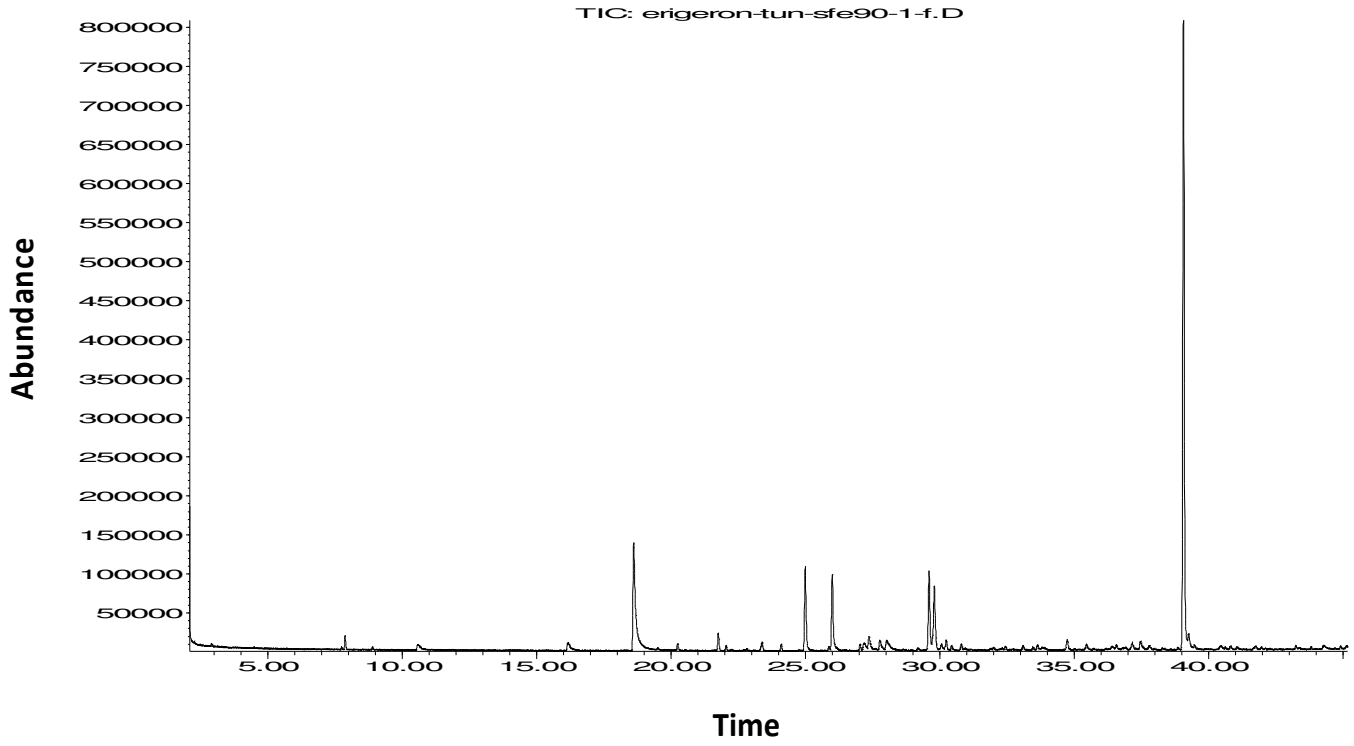

Figure 3. The GC/MS chromatogram of *Erigeron bonariensis* aerial parts collected from Monastir, Tunisia (essential oil prepared by SFE).

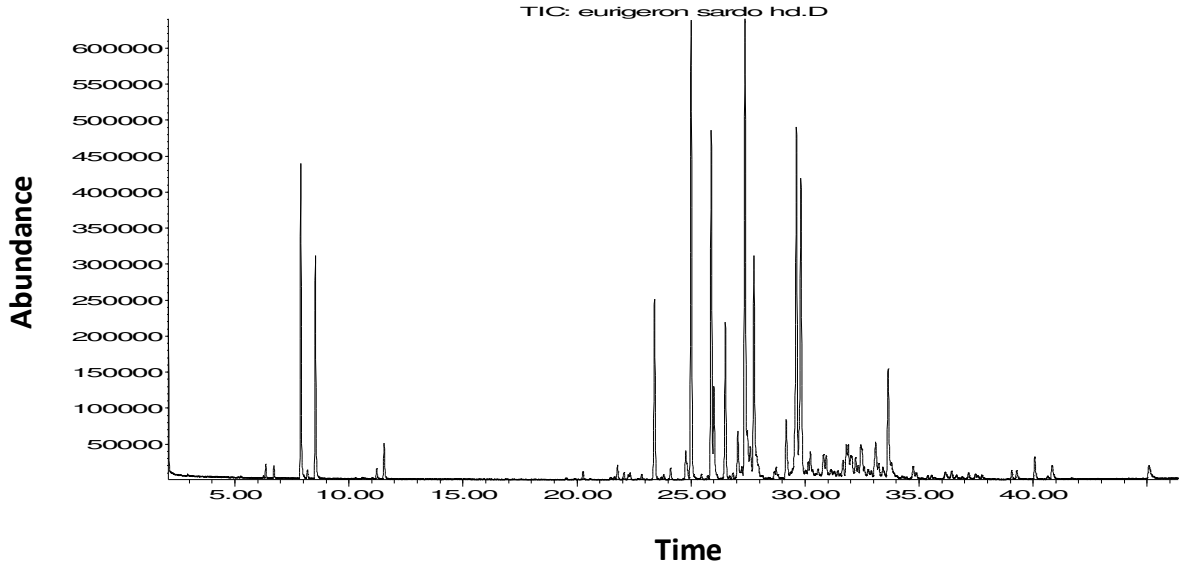

Figure 4. The GC/MS chromatogram of *Erigeron bonariensis* aerial parts collected from Cagliari, Sardinia (essential oil prepared by HD).

oil contained a higher level of sesquiterpenes (63.8%) comparing with that of Italian population (Table 2). Caryophyllene oxide (18.7%), spathulenol (18.6%) and α-curcumene (10.2%) have been detected as the

Table 2. composition of *E. bonariensis* aerial parts oils isolated by hydrodistillation (HD), the percentages are based on GC peak areas (effects of environmental conditions).

Retention time (min)	Retention idex (I_R)	HD (%) (Tunisia)	HD (%) (Sardinia)	Compound	Identification
2.86	800	0.2	-	n-octane	MS, RI
7.88	1026	5.1	6.5	limonene	MS, RI
8.53	1050	-	4.8	(E)-β-ocimene	MS, RI
10.53	1103	3.5	-	n-nonal	MS, RI
11.54	1132	-	-	(E,E)-2,6-dimethyl-1,3,5,7-octatetraene	MS, RI
16.13	1241	1.4	-	carvone	MS, RI
18.60	1300	9.8	-	carvacrol	MS, RI
20.25	1340	0.7	-	silphinene	MS, RI
21.76	1376	1.6	-	modheph-2-ene	MS, RI
22.05	1383	0.5	-	α-isocomene	MS, RI
23.39	1416	0.9	5.0	(E)-caryophyllene	MS, RI
24.77	1453	-	1.1	α-humulene	MS, RI
24.99	1456	4.0	12.0	(E)-β-farnesene	MS, RI
25.88	1480	-	9.7	γ-muurolene	MS, RI
26.00	1481	10.2	2.9	α-curcumene	MS, RI
26.50	1494	-	4.6	bicyclogermacrene	MS, RI
27.04	1507	1.4	-	α-bulnesene	MS, RI
27.36	1516	1.0	14.2	cis-lachnophyllum ester	MS, RI
27.75	1526	1.0	5.8	matricaria ester	MS, RI
29.17	1563	1.0	2.0	(E)-nerolidol	MS, RI
29.64	1575	18.6	11.4	spathulenol	MS, RI
29.82	1579	18.7	10.0	caryophyllene oxide	MS, RI
30.23	1590	1.9	0.5	salvial-4(14)-en-1-one	MS, RI
30.43	1595	0.5	-	syn-anti-anti-helifolen-12-al A	MS, RI
30.80	1605	2.0	0.6	humulene epoxide II	MS, RI
31.80	1634	-	0.8	caryophylla-4(12),8(13)-dien-5α-ol	MS, RI
31.90	1635	0.3	1.0	isospathulenol	MS, RI
32.44	1650	0.7	0.3	α-cadinol	MS, RI
33.63	1682	0.8	3.2	germacra-4(15),5,10(14)-trien-1-α-ol	MS, RI
39.06	1838	6.1	-	neophytadiene	MS, RI
Total identified		91.9	96.4	-	
Terpenes		86.2	76.4	-	
Monoterpenes		16.3	11.3	-	
Diterpenes		6.1	-	-	
Sesquiterpenes		63.8	29.8	-	

mostabundant constituents of Tunisian *E. bonariensis* essential oil while *Cis*-lachnophyllum ester (14.2%) was the major constituent of Italian (HD) volatile oil followed by (*E*)-*β*-farnesene (12.0%), spathulenol (11.4%) and caryophyllene oxide (10.0%).

DISCUSSION

Significant difference between the CO_2 SFE and the hydrodistillation processes is notified. Mainly, the diterpene neophytadiene (53.2%) detected as the chemo-type (main predominant terpene constituent) of Tunisian *E. bonariensis* volatile oil produced by supercritical carbon dioxide extraction is detected in lower amount in the distilled oil (6.1%). Thus, SFE is considered as an innovative method of volatiles extraction having a better selectivity for fragrant components. Furthermore, a marked difference between the qualities of HD essential oils of *E. bonariensis* growing in Tunisia and in Italy

(Table 2) is justified by the fact that the soil, the climate and the ecological conditions are important factors affecting the plant growth, the yield of extraction and eventually its medicinal properties. Although comparison of our results with those of Mabrouk et al. (2011) showed an agreement in herbal contents. Leaves oil previously prepared in summer by Mabrouk et al. (2011) was rich in caryophyllene oxide (22.6%), in spathulenol (15.1%) and in limonene (6.7%), while traditionally HD sample prepared in the current study contains 18.7, 18.6 and 5.1% of the same compounds, respectively. The difference in percentages indicates that the quantity of active materials in leave Tunisian *E. bonariensis* essential oil was more than that of the whole plant. High proportions of main components caryophyllene oxide and spathulenol in *E. bonariensis* HD essential oil suggest that the specie can be an excellent source of both bioactive substances which may contribute to its pharmacological properties. Caryophyllene oxide was reported to act as a potent anti-carcinogenic agent exhibiting cytotoxic effects against several tumor cell lines (Park et al., 2011) and spathulenol have been previously cited for its moderate activity against strains of human carcinogenic cells (Mendes et al., 2008).

Conclusion

This paper presented the first study of the effects of the variability of ecological factors and extraction techniques on the quality of volatiles from *E. bonariensis* species. Thus essential oils were extracted using traditionally HD and innovative SFE methods. The chemical composition analysis based on GC/MS showed that SFE have a better selectivity for fragrant constituents. Furthermore, comparison of essential oils extracted from both Tunisian and Sardinian *E. bonariensis* showed that variability of climatic and soil conditions affect the chemical composition as well as the yield of extraction.

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