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Full Length Research Paper

Chemical composition of volatile compounds and antioxidant activities of essential oil, aqueous and ethanol extracts of wild Tunisian *Ruta chalepensis* L. (Rutacea)

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The chemical composition of the volatile fractions of two populations of *Ruta chalepensis* L. (Rutaceae) collected, at the vegetative stage, from two areas of the South-East of Tunisia (Sdira and Thoujene) were analysed. The volatile fractions were extracted from the leaves by hydrodistillation or static headspace, then analysed by gas chromatography flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS). Octyl acetate (24.22 to 33.16 %), 2-undecanone (12.43 to 23.82%) and 2-nonanone (14.11 to 41.69%) were found to be major components of the volatiles extracted by hydrodistillation or head space method. Minor components revealed by hydrodistillation were not detected by head space extraction. However, hexadecanoic acid (10.2%) and di(ethylhexyl) phthalate (12.73%) were detected only in headspace of *R. chalepensis* leaves from Thoujene. Unlike the essential oil, the ethanol and the aqueous extracts revealed interesting antioxidant activities including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, reducing power and metal (Fe^{2+}) chelating activity and could be considered as important sources of natural antioxidants.

Key words: *Ruta chalepensis*, hydrodistillation, headspace, ketones, esters, antioxidant activity.

INTRODUCTION

Essential oils and various extracts obtained from many plants have recently been gaining a growing popularity and scientific interest. In fact, the interest in naturally occurring antioxidants has considerably increased in various products to replace synthetic antioxidants which are being restricted due to their carcinogenicity. Consequently, the commercial development of plants as new sources of antioxidants to enhance health and food preservation is of prime importance. *Ruta chalepensis* L. is an aromatic plant belonging to the Rutaceae family, commonly named by local population as "Fidjel". It's a perennial herb widely spread in the Mediterranean area and usually growing on rocky slopes (Iauk et al., 2004). *R. chalepensis* is characterised by glabrous, alternate bi-pennatisect leaves, narrow oblong-lanceolate or obovate segments and cymose inflorescence. Oil glands are principally present in leaves, having a strong deterrent odour. *R. chalepensis* is one of the most frequently used plants in the folk medicine for its emmenagogue, antihelmintic, anti-inflammatory and spasmolytic effects (Di-Stasi et al., 1994; Atta and Alkofahi, 1998). In Saudi Arabia, a decoction of the aerial parts of the plant is used as an analgesic and antipyretic and for the treatment of

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rheumatism and mental disorders (Iauk et al., 2004). The leaves of this plant infused with vinegar are given to children for the treatment of convulsion and other nervous disorders. In fact, it was shown that ethanol extract of the aerial parts of *R. chalepensis* produced a significant central nervous system depressant activity in mice (Gonzalez-Trujano et al., 2006).

Furthermore, it was also shown that the aqueous extract of the *R. chalepensis* leaves had a spermotrophic action demonstrated by the increase in sperm count, motility, living percent, and decrease in encountered sperm abnormalities. The hormonal profile was also influenced since the testosterone and follicle stimulating hormone levels were significantly increased with no change in the leutinizing hormone and prolactin levels (Al Qarawi, 2005).

The chemical composition of essential oils depends on a large number of parameters, namely, climatic, seasonal and geographic conditions, harvest period and extraction technique, among others. Indeed, knowledge of the chemical composition of essential oils is a very important quality criterion for their marketing and contributes to their valorization. The chemical composition of essential oils of different aerial parts of *R. chalepensis* plants grown at different locations have shown wide range of variations even in their major constituents (Baser et al., 1996; Rustaiyan et al., 2002; Bagchi et al., 2003; Saidani-Tounsi et al., 2011; Ntalli et al., 2011). Few studies investigated the chemical composition of *R. chalepensis* essential oil from North Africa. Furthermore, published literature did not report antioxidant activities of *R. chalepensis* extracts.

Therefore, the aim of the present work is to provide more information on the chemical composition of the essential oil obtained by hydrodistillation of *R. chalepensis* leaves originated from two provenances in the South region of Tunisia. Furthermore, a technique based on static headspace extraction of volatiles coupled with GC/MS analysis was also applied to evaluate the major volatile compounds. The antioxidant activities of essential oil, ethanol and aqueous extracts were also investigated by DPPH, reducing power and metal (Fe^{2+}) chelating assays. Additionally, the total phenolics and flavonoids contents of these extracts have been determined.

MATERIALS AND METHODS

Chemicals

DPPH, butyl-hydroxytoluene (BHT), ethylenediamine tetraacetic acid (EDTA), gallic acid and quercetin were from Sigma Chemical (St. Louis, USA). All other chemicals were of analytical grade.

Plant material

The aerial parts of three individuals of *R. chalepensis* L. spaced at a distance of about 20 m were collected from two sites located at the South-East of Tunisia with an arid climate characterized by a mean rainfall of 150 mm/year: Sdira (33° 18′ 35″ N, 10° 12′ 2″ E at 284 m altitude) and Thoujene (33° 27′ 34″ N, 10° 8 ′ 17″ E at 525 m altitude). All samples were collected at the vegetative stage (December 2010) and identified according to the ‘‘Flore de la Tunisie’’ (Chaieb and Boukhris, 1998). The fresh vegetable matter was dried in the shadow, until constancy of the weight (20 days). Finally, leaves were separated from stems and subjected for volatile compounds extraction and preparation of aqueous and ethanol extracts.

Volatile compounds extraction

Two different extraction techniques were used to investigate the volatiles, including hydrodistillation (HD) and static headspace (HS) extraction with subsequent analysis by GC/FID and GC/MS.

Essential oil extraction by hydrodistillation

The dry matter was submitted to hydrodistillation for 4 h, using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from light at -20°C until analysis.

Volatiles extraction by static HS

One of the drawbacks of hydrodistillation is that it is a time-consuming method and large amounts of plant material are needed. Therefore, static headspace extraction which is a rapid technique of gas extraction (Snow and Slack, 2002) was applied and compared to hydrodistillation extraction. Agilent Technologies G1888 headspace auto-sampler coupled with an Agilent Technologies 6890N GC and an Agilent Technologies 5973B MS was used. One gram of each leaves sample was introduced into 20 ml HS vial and sealed using silicone septa and aluminum foil. The vial was then thermostated at 45°C±0.1. The volatile compounds migrate out of the leaves into the empty space of the bottle. After the equilibrium was established (30 min), 1 ml of vapor generated from the above vegetal was drawn out from the vial using a gas-tight syringe (60°C) and injected directly in the chromatographic column *via* a transfer line (75°C).

Volatile compounds analysis

Gas chromatography (GC)

An Agilent Technologies 6890N GC equipped with HP-5MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm; Hewlett-Packard) and connected to a FID was used. The column temperature was programmed at 50°C for 1 min, then 7°C/min to 250°C, and then left at 250°C for 5 min. The injection port temperature was 240°C; while of the detector was 250°C (split ratio: 1/60).

The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min. The analysed sample volume was 2 µl of essential oil or 1 ml of vapor generated from HS extraction. Percentages of the constituents were calculated by electronic integration of FID peak areas, without the use of response factor correction. Mean percentage of volatiles compounds in *R. chalepensis* represented the average calculated on three individuals. Retention indices (RI) were calculated for separate compounds relative to C_8-C_{26} n-alkanes mixture (Aldrich Library of Chemicals Standards) (Kovàts, 1958).

Gas chromatography/Mass spectrometry (GC/MS)

The isolated volatile compounds by HS or HD were analysed by GC/MS, using an Agilent Technologies 6890N GC. The fused HP-5MS capillary column (the same as that used in the GC/FID analysis) was coupled to an Agilent Technologies 5973B MS (Hewlett-Packard, Palo Alto, CA, USA). The oven temperature was programmed at 50°C for 1 min, then 7°C/min to 250°C, and then left at 250°C for 5 min. The injection port temperature was 250°C and that of the detector was 280°C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min. The MS conditions were as follow: ionization voltage, 70 eV; ion source temperature, 150°C; electron ionization mass spectra were acquired over the mass range 50 to 550 m/z.

Volatile compounds identification

The volatile compounds of *R. chalepensis* leaves were identified by comparing the mass spectra data with spectra available from the Wiley 275 mass spectra libraries (software, D.03.00). Further identification confirmations were made referring to RI data generated from a series of known standards of n-alkanes mixture (C_8 to C_{26}) (Kovàts, 1958) and to those previously reported in the literature (Adams 2001; Demetzos et al., 2002; Shang et al., 2002; Mimica-Dukic et al., 2003; Asuming et al., 2005; Pino et al., 2005; Sajjadi and Eskandari, 2005; Setzer et al., 2005; Kallio et al., 2006; Hazzit et al., 2006; Radulovic et al., 2007; Zouari et al., 2010).

Preparation of *R. chalepensis* extracts

The dried powder of the *R. chalepensis* leaves (30 g) was Soxhlet-extracted with 250 ml ethanol during 6 h. Then, the solvent was evaporated using a rotary evaporator and the residual solvent was removed by flushing with nitrogen. Finally, the obtained extract was kept in the dark at + 4°C until further analysis. For the aqueous extract, the powdered *R. chalepensis* leaves (40 g) were macerated with 600 ml distilled water at ambient temperature for 3 days using a shaker at a constant stirring rate (200 rpm). Afterwards, the solids were separated by filtration and the solution was lyophilized.

Total phenolic and total flavonoid content

The total phenolic content of *R. chalepensis* was determined by the Folin-Ciocalteu method (Dewanto et al., 2002). Gallic acid monohydrate was used as standard for the calibration curve. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/g extract.

The total flavonoid content of the samples was determined as previously described (Dewanto et al., 2002) and quercetin was used as standard. The results were expressed as mg quercetin equivalent (QE)/g extract.

Antioxidant activity

The metal (Fe^{2+}) chelating activity, the reducing power and the DPPH radical-scavenging activity of *R. chalepensis* extracts were measured as previously described (Dinis et al., 1994; Yildirim et al., 2001; Zouari et al., 2011a).

Statistical analysis

Values were expressed as means ± standard deviation. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA with a SPSS 11 (Statistical Package for the Social Sciences) programme. Differences at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Chemical composition of volatile compounds

The volatile compounds of wild *R. chalepensis* leaves collected from two locations of the South-East of Tunisia were investigated using two different extraction techniques, including HD and HS extraction, in association with GC/FID and GC/MS. The components identified for each location (Sdira: RC1 and Thoujene: RC2), their percentages and their RI are listed in Table 1 in order of their elution on the HP-5MS column. The HD technique resulted in the identification of more compounds as compared to the HS method. From the data obtained, a total of sixteen compounds were identified in the essential oils of RC1 and RC2. However, using HS extraction, only seven compounds were identified in RC1 and ten compounds in RC2, accounting for 99.15 and 95.47% of total volatiles, respectively (Table 1). The chemical classes' distributions of *R. chalepensis* volatile compounds were also reported in Table 1. All identified compounds were separated on the basis of their chemical structures into 7 classes, which were ketones, esters, acids, alcohols, aldehyds, hydrocarbons and diterpenes. Whatever the plant location or the extraction method, we note that the compositions of the investigated samples were dominated by ketones (37.49 to 57.92%) and esters (38.86 to 44.55%).

A survey of the literature shows that essential oils of different aerial parts of *R. chalepensis* plants grown at different locations have shown wide range of variations even in their major constituents. In fact, a composition dominated by 2-undecanone was found in the essential oil of *R. chalepensis* from Turkey (66%), Iran (52%) and India (41 to 68%). However, essential oil of this plant from Italy showed that 2-undecanone and 2-nonanone were major compounds. Moreover, 2-undecanone (26 to 44%) and 2-nonanol (28 to 40%) were found to be the main compounds in essential oils of growing wild *R. chalepensis* from the center of Tunisia (Baser et al., 1996; Rustaiyan et al., 2002; Bagchi et al., 2003; Ntalli et al., 2011; Saidani-Tounsi et al., 2011). By contrast, our results clearly indicate the occurrence of a new chemotype of *R. chalepensis* growing wild in the South-East (Sdira and Thoujene) of Tunisia containing octyl acetate (28.5 to 33.16%), 2-undecanone (22.6 to 23.8%) and 2-nonanone (14.1 to 16.97%) as the major constituents of the essential oil. It is also to note that other fonctionalised compounds such as isomaturnin was detected for the first time in the *R. chalepensis* essential oil. Esters, ketones, acids and alcohols are the principles

Table 1. Mean percentage of volatile compounds of *R. chalepensis* using essential oil extraction by hydrodistillation followed by GC/MS analysis (HD/GC/MS) or static headspace extraction of volatiles coupled with GC/MS analysis (HS/GC/MS).

S/N	Compounds[a]	RI[b]	HD/GC/MS		HS/GC/MS		Identification[d]
			RC1[c]	RC2[c]	RC1[c]	RC2[c]	
1	*Trans*-2-Hexenal	849	0.10	0.12	-	-	RI, MS
2	2-Octanone	986	-	0.10	-	-	RI, MS
3	2-Octanol	997	-	0.10	-	-	RI, MS
4	2-Nonanone[e]	1088	16.97	14.11	41.61	24.38	RI, MS
5	2-Nonanol	1096	2.48	4,29	2.37	3.57	RI, MS
6	2-Octyl acetate	1132	1.50	1.03	1.93	0.58	RI, MS
7	Geijerene	1137	0.83	0.55	-	-	RI, MS
8	2-Decanone	1185	1.64	1.49	1.24	0.68	RI, MS
9	Octyl acetate[e]	1232	33.16	28.61	32.88	24.22	RI, MS
10	2-Undecanone[e]	1288	23.82	22.59	15.07	12.43	RI, MS
11	2-Dodecanone	1384	0.58	1.38	-	-	RI, MS
12	Decyl acetate	1422	9.89	10.48	4.05	4.79	RI, MS
13	2-Tridecanone	1485	0.64	0.91	-	-	RI, MS
14	Hexadecene	1581	-	-	-	1.89	RI, MS
15	3,4-Methylenedioxybenzylacetone	1592	0.24	0.27	-	-	MS
16	Hexadecanoic acid[e]	1964	-	-	-	10.2	RI, MS
17	Phytol	2098	-	0.11	-	-	RI, MS
18	Isomaturnin	2173	1,18	2.64	-	-	MS
19	Di(ethylhexyl) phthalate[e]	2519	-	-	-	12.73	RI, MS
	Total identified (%)		93.03	88.78	99.15	95.47	
	Grouped components						
	Hydrocarbons		0.83	0.55	-	1.89	
	Alcohols		2.48	4.39	2.37	3.57	
	Aldehyds		1.28	2.76	-	-	
	Ketones		43.89	40.85	57.92	37.49	
	Esters		44.55	40.12	38.86	42.32	
	Acids		-	-	-	10.2	
	Diterpenes		-	0.11	-	-	

[a]Compounds are listed in order of their elution from a HP-5MS column and their percentages were obtained by FID peak-area normalization. [b]RI, retention indices calculated against C_8–C_{25} n-alkanes mixture on the HP 5MS column. [c]RC1, RC2, *R. chalepensis* from Sdira at 284 m altitude and Thoujene at 525 m altitude, respectively (South of Tunisia). [d]Identification: RI, comparison of retention indice with bibliography; MS, comparison of mass spectra with MS libraries. [e]Major compound in bold font.

of the mycofumigation effects and act also among others against phytonematodes. In fact, 2-undecanone as well as *R. chalepensis* essential oil has been reported to exhibit high nematicidal activity against both nematodes *Meloidogyne incognita* and *Meloidogyne javanica* (Ntalli et al., 2011). Besides, 2-undecanone was reported to act strongly as a repellent against *Tetranychus urticae* considered as a pest (Antonious and Snyder, 2006) as well as against arthropods, and therefore it is formulated in domestic-use insect repellents (Bissinger et al., 2009).

Time, cost, ease of operation and possibility of analysis of volatiles in solid as well as in liquid samples are the main advantages of the HS technique as compared to hydrodistillation extraction. In fact, HS extraction was carried out using small amount of leaves and short total analysis time as compared to the traditional technique of hydrodistillation. However, it is known that HS technique is essentially a method of analyzing the very volatile components. These two extraction techniques show different profiles of chemical compositions (Table 1). Although, minor components revealed by HD were not detected by HS extraction, octyl acetate, 2-undecanone and 2-nonanone remain major components in both techniques. Nevertheless, it is surprising to note that two other compounds with relatively high percentages such as hexadecanoic acid (10.2%) and di(ethylhexyl) phthalate (12.73%) were detected only in headspace of the *R. chalepensis* leaves from Thoujene (RC2) (Table 1).

Different profiles of volatile compounds detected by HD or HS may be related to the operating conditions (for

Table 2. Extraction yields, total phenolic and total flovonoid contents of essential oil, ethanol and aqueous extracts from *R. chalepensis*.

	Essential oil		Aqueous extract		Ethanol extract	
	RC1	RC2	RC1	RC2	RC1	RC2
Yield[a] (%)	2.32±0.2^{A}	1.25±0.07^{B}	9.27±0.2^{A}	11.85±0.15^{B}	14.86±0.24^{A}	18.06±0.3^{A}
TPC[b] (mg GAE/g extract)	0.0	0.0	151.28±4.9^{A}	149.09±4.93^{A}	54.13±1.42^{A}	67.23±2.75^{B}
TFC[c] (mg QE/g extract)	0.0	0.0	87.12±3.22^{A}	71.80±4.97^{B}	347.33±7.95^{A}	374.70±5.53^{A}

Data presented as the mean ± standard deviation (n = 3). [a]Yields of essential oil expressed in ml/100 g dry weight and Yilelds of aqueous and ethanol extracts expressed in g/100 g dry weight; [b]Total phenolic content as gallic acid equivalent; [c] Total flavonoid content as quercetin equivalent. In each row, different uppercase superscript letters indicate significant differences (p<0.05).

example, the sampling temperature and time). In fact, essential oils components can be modified during the hydrodistillation process. Our results show that in the case of *R. chalepensis*, HS analysis is suitable for detecting volatile compounds from leaves that can be altered by the traditional technique of HD and therefore it gives the most natural and true picture of the composition of volatiles.

Antioxidant potential

Phenolics and flavonoids contents

The yields of extractable compounds relative to the weight of dried plant leaves ranged from 9.27 to 11.85% (w/w) for the aqueous extract and from 14.86 to 18.06% (w/w) for the ethanol extract. Moreover, essential oil yields of *R. chalepensis* from Sdira and Thoujene were found to be 2.32 and 1.25% (v/w), respectively (Table 2). Interestingly, the essential oil yields were relatively high as compared with that of the essential oil of *R. chalepensis* collected from Italy at the same stage (0.36%) (w/w) (Ntalli et al., 2011). It is well known that phenolic substances such as flavonoids, phenolic acids, and tannins contribute directly to the antioxidant capacity of plants. In fact, phenolic compounds exhibit considerable free radical-scavenging activities (through their reactivity as hydrogen- or electron-donating agents) and metal ion-chelating properties, preventing metal-induced free radical formation (Rice-Evans et al., 1996).

Furthermore, these antioxidants may also contribute to diverse biological activities such as anti-inflammatory, anti-atherosclerotic and anticarcinogenic activities (Chung et al., 1998). Therefore, the amounts of total phenolics and flavonoids in these extracts were determined (Table 2). Our results showed that the aqueous extract had the highest phenolics content, whereas the ethanol extract had the highest flavonoids content. Unlike the ethanol and the aqueous extracts, essential oil of *R. chalepensis* did not contain phenolic compounds and this was also shown by its chemical composition analysis (Table 1).

DPPH radical-scavenging activity

Essential oil, aqueous and ethanol extracts of *R. chalepensis* leaves were subjected to screening for their possible DPPH radical-scavenging activities (Figure 1a). The effect of antioxidant on DPPH radical-scavenging was thought to be due to their hydrogen donating ability. When a solution of DPPH is mixed with that of a substance, it can generate a hydrogen atom. This results in the reduced form of DPPH (non-radical) with the loss of the violet color. DPPH scavenging activity is usually presented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Therefore, extract concentrations providing 50% inhibition (IC_{50}) were calculated using the data plotted in Figure 1a. Lower IC_{50} value reflects better DPPH radical-scavenging activity. Aqueous and ethanol extracts were able to effectively reduce the stable free radical DPPH with an IC_{50} values ranging from 0.12 to 0.22 mg/ml, but not stronger than the standard BHT (IC_{50} = 25 µg/ml) (Figure 1a). These results suggest that the presence of phenolic compounds in aqueous and ethanol extracts may be the main cause of their considerable radical-scavenging activity (Table 2).

For comparative purposes, ethanolic leaves extract of *R. chalepensis* showed stronger DPPH radical-scavenging activity than that of *Ruta graveolens* (19.37% of inhibition at 0.4 mg/ml) (Pandey et al., 2011). Nevertheless, the essential oil of *R. chalepensis* leaves did not display a noteworthy DPPH-radical scavenging activity. This result is expected because the chemical composition of *R. chalepensis* essential oils shows an absence of phenolic or oxygenated terpenic compounds and a dominance of aliphatic ketones and esters components (Table 1). Essential oils not containing phenolics, but rich in oxygenated monoterpenes have relatively important DPPH scavenging properties (Zouari et al., 2011a). Literature is scare on the antioxidant activities of aliphatic ketones and esters as major components of the plant essential oil. However, essential oil of *Ruta montana* containing 2-undecanone (32.8%), 2-nonanone (29.5%) and 2-nonanol acetate (18.2%) as major compounds, show weak DPPH radical-scavenging activity (IC_{50} value = 16.7 µl/ml) (Kambouche et al., 2008).

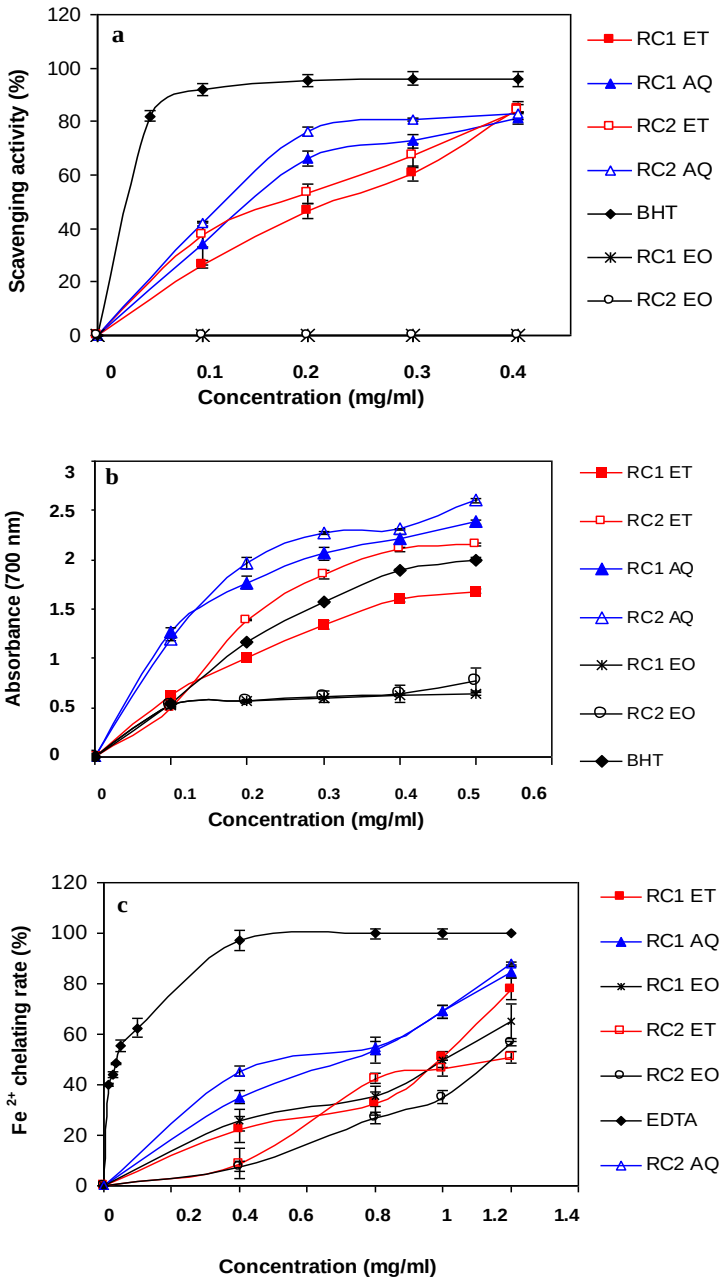

Figure 1. Antioxidant activity of *R. chalepensis* (RC) essential oils (EO), ethanol extracts (ET) and aqueous extracts (AE). DPPH-scavenging activity; **a**, reducing power; **b** and metal (Fe^{2+}) chelating activity; **c**. BHT was used as positive control in DPPH and reducing power assays. EDTA was used as positive control in metal chelating assay. RC1, RC2, *R. chalepensis* from Sdira at 284 m altitude and Thoujene at 525 m altitude, respectively (South-East of Tunisia).

Reducing power

In the reducing power assay, the presence of antioxidants in the sample would result in the reducing of Fe^{3+}–Fe^{2+} by donating an electron. An amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue ($Fe_4[Fe(CN)_6]_3$) at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 1b showed the reducing power of essential oil, aqueous and ethanol extracts as compared with BHT as standard. It was found that the reducing power of aqueous and ethanol extracts

increased with the increase of their concentrations. The aqueous extract, which contained the highest amount of total phenolics, showed a higher reducing power than ethanol extract and synthetic BHT. Besides, the aqueous and the ethanol extracts showed a much more reducing power than essential oil which did not contain any phenolics (Figure 1b). These results are in agreement with the fact that Fe^{3+} reduction is an important mechanism of phenolic antioxidant action (Yildirim et al., 2001; Zouari et al., 2011b).

Metal (Fe^{2+}) chelating power

Metal chelating activity is claimed as one of antioxidant mechanisms, since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. Among the transition metals, Fe^{2+} ion is known as the most important lipid oxidation prooxidant due to its high reactivity (Liu et al., 2007). The dark color of complex formed by the interaction of ferrozin with Fe^{2+} ions is decreased by the action of metal chelator compounds that existed in the reaction mixtures. Data presented in Figure 1c show the chelating activity of *R. chalepensis* extracts at different concentrations (0.4 to 1.2 mg/ml) and compared with EDTA as positive standard. The results showed that the percentages of inhibition of the ferrozine–Fe^{2+} complex formation increased with increasing concentration of the extracts. The aqueous extract also showed the highest metal chelating activity (IC_{50} values = 0.52 to 0.74) when compared to essential oil or ethanol extract (IC_{50} values = 1 to1.2), but not stronger than the standard EDTA. Although the chemical EDTA exhibited the highest metal chelating activity (IC_{50} value = 0.04), natural antioxidants remain of growing interest.

Conclusions

This study is a contribution to the chemical and biological studies of Tunisian flora and within the aim of valorising medicinal *R. chalepensis* widely growing in the country. Our results allowed the identification of new chemotype of *R. chalepensis* growing wild in the South-East of Tunisia (Sdira and Thoujene) containing octyl acetate, 2-undecanone and 2-nonanone as the major components of the volatiles extracted by hydrodistillation or head space method. Nevertheless, two other compounds with relatively high percentages such as hexadecanoic acid and 1,2-benzene dicarboxylic acid, bis (2-ethylhexyl) ester were detected only in headspace of the *R. chalepensis* leaves. Unlike the essential oil, the aqueous or the ethanol extracts of *R. chalepensis* may be suggested as new potential sources of natural antioxidants in food and pharmaceutical industries. These extracts were found to be effective antioxidants in different *in vitro* assays including DPPH radical-scavenging activity, reducing power and metal (Fe^{2+}) chelating power. This study needs to be continued to other phenological stages for a better phytochemical characterization of *R. chalepensis* growing wild in Tunisia in order to improve its rational uses.

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