African Journal of Biotechnology Vol. 9(18), pp. 2709-2716, 3 May, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1612
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Phenols, essential oils and carotenoids of *Rosa canina* from Tunisia and their antioxidant activities

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Accepted 26 February, 2010

The antioxidant activity of leaf extracts of *Rosa canina* from diverse localities of Tunisia were evaluated by ABTS and DPPH methods, whereas in those of essential oils and carotenoids extracts such activity was determined only by the ABTS method. Total phenols determined by the Folin method revealed that at Aindraham, samples showed a great variability of phenol content in contrast to those from Feija. After chemical analysis of the essential oils by gas chromatography (GC) and gas chromatography coupled to mass spectra (GC-MS), revealed that the oils of Feija were predominantly composed of palmitic acid, vitispirane, linoleic acid, lauric acid, myristic acid and phytol acetate, while in those samples from Aindraham predominated vitispirane, palmitic acid, linoleic acid and phytol acetate. Higher concentrations of β-carotene and lycopene were found in the samples from Aindraham after determination by high performance liquid chromatography (HPLC). All samples possess antioxidant activity, nevertheless much more significant in phenol extracts in contrast to the carotenoid extracts, which possess the lowest activity.

Key words: Secondary metabolites, biological activity, natural products.

INTRODUCTION

The genus *Rosa*, with over 100 species, is one of the most widespread members of the Rosacea family. This genus is widely distributed in Europe, Asia, the Middle East and North America (Nilsson, 1997). In Tunisia, eight species of this genus have been described by Alapetite 1979): *Rosa gallica L., R. agrestis, R. sicula, R.* (*semper - virens*, *R. stylosa* (Desv)*, R. micrantha*, *R. canina* and *R. moschata.*

R. canina (dog rose) is an erect shrub of up to 3.5 m, sometimes climbing; branches often curved or arched. Petals are white to pale pink, rarely deep pink and fruit ripens late (Ercisli, 2005). Fruits (hips) have long been traditionally used in the prevention and therapy of common cold and other infections, as diuretic agent and for the treatment of various inflammatory diseases. So far, none of these indications clinical effectiveness has been demonstrated except for osteoarthritis (Chrubasik et al., 2006; Christensen et al., 2008; Chrubasik et al., 2008; Kharasmi, 2008; Winther, 2008).

According to the German Commission E Monographs, fruits (rose-hips, with seeds) of *R. canina* L. possess

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Abbreviations: DPPH, 2,2'-Diphenyl-1-picrylhydrazyl radical; **ABTS,** 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; **GC,** gas chromatography; **GC-MS,** gas chromatography coupled to mass spectra; **HPLC,** high performance liquid chromatography; **MRSA,** methicillin-resistant *Staphylococcus aureus*; **IC_{50},** sample concentration providing 50% inhibition; **SWE,** super-heated water extraction.

prophylactic and therapeutic activities against inflammatory disorders including arthritis, rheumatism, gout and sciatica and can be used for diseases with fever, for colds and influenza, for the prevention of inflammation of the gastric mucosa and gastric ulcer, for gallstones, biliary complaints, as a laxative, for disorders of the kidney and the lower urinary tract, as a diuretic, for dropsy and as an astringent (Orhan et al., 2007; Wenzig et al., 2008; Fecka, 2009).

Antioxidant activity of 50% aqueous methanol extracts of *R. canina* has also been reported, mainly as free radical scavenging and hydrogen peroxide scavenging activities (Serteser et al., 2008). Recent studies revealed that *R. canina* extracts were effective on the inhibition of growth and biofilm formation in methicillin-resistant *Staphylococcus aureus* (MRSA) (Quave et al., 2008).

Such activities can be attributed to the different components present in this species: phenols, carotenoids, vitamin C, tocopherol, pectin, sugar, organic acids, amino acids and essential oils (Ercisli, 2007). However, several factors including genotype, climate, region, harvesting time and altitude, may be responsible for diverse chemical composition and consequently activities.

The purpose of this work, which is carried out for the first time in Tunisie, is to characterize chemically *R. canina* leaves and pericarps collected in different localities through its essential oil and carotenoids composition as well as to evaluate their antioxidant activities.

MATERIALS AND METHODS

Plant material

The identification of plant materials was confirmed by Prof. Dr Hasnaoui Brahim, in the Departement of Ecology, Sulvo-pastoral de Tabarka, Tunisie. Plants of *R. canina* were collected from Kroumirie at Feija and Aindraham, North Tunisia, during September - October 2008. The collective sample was constituted of a mixture of 10-15 individual plants.

Isolation of leaf essential oils

The dried leaves were subjected to hydrodistillation (100 g) for 3 h, using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate before analysis. The oil yields were 0.2 and 0.1% (v/w) for samples harvested in Feija and Aindraham, respectively.

Preparation of the extracts

A portion of dried plant material (3 g) was extracted with hexane, followed by dichloromethane and methanol in a Soxhlet apparatus (6 for each solvent). Removal of the solvent was carried out by vacuum distillation at 40°C. The extracts were kept in the dark at -4°C until use.

Extraction of pericarp carotenoids

Carotenoid extraction was carried out as previously described by Hodisan et al. (1997) with some modifications. Briefly, pericarps of *R. canina* were powdered into liquid nitrogen. The powder was immediately extracted with acetone-methanol-petroleum ether (3: 2: 1, v: v: v) for 5 h in the dark. Thereafter, the extract was filtered and evaporated to dryness in a rotavapor and ressuspended in ethyl ether. The ether solution was then partitioned with water. An equal volume of 30% (w/v) KOH in methanol was added to the ether fraction and saponification was completed overnight at 5°C with magnetic stirring. The unsaponifiable components were washed out with water until the washings were at neutral pH. The total carotenoid extracts were evaporated at 10°C in a rotary evaporator. The dried pigments were dissolved in dichloromethane and analysed by HPLC.

Essential oils analysis

Gas Chromatography (GC)

Gas chromatographic analyses were performed using a Perkin Elmer Autosystem XL gas chromatograph equipped with two flame ionization detectors (FIDs), a data handling system and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m x 0.25 mm i. d., film thickness 0.25 μm) (J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30 m x 0.25 mm i. d., film thickness 0.15 μm) (J & W Scientific Inc.). Oven temperature was programmed, 45 - 175°C, at 3°C/min, subsequently at 15°C/min up to 300°C and then held isothermal for 10 min; injector and detector temperatures, 280°C and 300°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using split sampling technique, ratio 1:50. The volume of injection was 0.2 μL of a pentane-oil solution. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each oil, without using correction factors.

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS unit consisted of a Perkin Elmer Autosystem XL gas chromatograph, equipped with DB-1 fused-silica column (30 m x 0.25 mm i. d., film thickness 0.25 μm) (J & W Scientific, Inc.) and interfaced with a Perkin-Elmer Turbomass mass spectrometer (software version 4.1). Injector and oven temperatures were as above; transfer line temperature, 280°C; ion trap temperature, 220°C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1: 40; ionization energy, 70 eV; ionization current, 60 μA; scan range, 40 - 300 u and scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C_9–C_{21} *n*-alkane indices and GC-MS spectra from a home-made library, constructed based on the analyses of reference oils, laboratory-synthesised components and commercial available standards.

Determination of total phenols

The total phenol contents in the hydrodistillation-aqueous phase extracts and Soxhlet extracts were determined using the Folin-Ciocalteu reagent and gallic acid as standard as described by Slinkard and Singleton, (1977). The sample (0.5 mL) and 2 mL of sodium carbonate (75 g/L) were added to 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm in a Shimadzu 160-UV (Tokyo, Japan) spectrophotometer. The results are given as gallic acid equivalent /mL extract (GAE/mL extract).

Determination of flavones and flavonols

The assay was performed according to that previously described by Popova et al. (2004) with some modifications. Briefly, 1 mL of plant extract or standard was mixed with 1 mL aluminium trichloride in methanol (2%) and the volume was made up to 25 mL with methanol. The mixture was left for 40 min and the absorbance at 420 nm was measured in a Shimadzu 160-UV (Tokyo, Japan) spectrophotometer. The results are given as rutin equivalent /mL extract.

HPLC analysis

Carotenoids were analysed as previously reported (Kozukue and Friedman, 2003). Briefly, separations were performed on a Purospher column RP-18 (250 mm x 4 mm; 5 μm particle size, Merck (USA)), using HPLC with a system Gold Programmable Detector Module 166-UV-Vis (Beckman Coulter, USA). The mobile phase was acetonitrile/methanol/dichloromethane/n-hexane (50: 40: 5: 5 v/v/v/v) at a flow rate of 1 mL/min. The volume of each injection was 20 μL using an injector with a 20 μL loop (Rheodyne, Calif, USA). Detection was performed at 453 nm.

Quantification of the carotenes was achieved by comparing sample peak areas with those of known amounts of the standards β-carotene and lycopene. These carotenoids were identified by comparing HPLC retention times with those of known standards.

Free radical scavenging activity (DPPH)

A methanolic stock solution (50 μL) of each sample (essential oils and extracts) was placed in a cuvette and 2 mL of 60 μM methanolic solution of DPPH was added (Brand-Williams et al., 1995). Absorbance measurements were made at 517 nm using a Shimadzu 160-UV spectrophotometer (Tokyo, Japan) after 3 min of reaction at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as negative control. The percentage inhibition of the DPPH radical by the samples was calculated according to the following formula: Scavenging effect % = $[(A_0 – A_1) / A_0] * 100$ where A_0 was the absorbance of the blank sample and A_1 was the absorbance in the presence of the sample. Tests were carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample (essential oil or extract solution) concentrations.

Free radical scavenging ability using ABTS radical cation ABTS

ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 24 h before use (Re et al., 1999). Afterwards, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.7 at 734 nm. After addition of 990 μL of diluted $ABTS^{\bullet+}$ solution to 10 μL of sample, the absorbance reading was taken at 30°C exactly 6 min after initial mixing, at 734 nm, using a Shimadzu 160-UV spectrophotometer (Tokyo, Japan). Absorption of a blank sample containing the same amount of ethanol and $ABTS^{\bullet+}$ solution acted as negative control. The percentage inhibition of the ABTS cation radical by the samples was calculated according to the following formula: *Scavenging effect % = $[(A_0 – A_1) / A_0] * 100$* where A_0 was the absorbance of the blank sample and A_1 was the absorbance in the presence of the sample. Tests were carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample (essential oil or extract solution) concentrations.

Statistical analysis

Results are reported as the mean ± standard deviation. Analysis of variance was performed by ANOVA procedures (SPSS 14.0 for Windows). Significant differences between means were determined by Tukey post hocs tests, *p* values inferior to 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Essential oils

The components present in the leaf oils of *R. canina* are listed in Table 1 in order of their elution on a DB-1 column. In samples from Feija, 83.2% of the total oil was identified, predominating palmitic acid (23.2%), vitispirane (9.1%), linoleic acid (7.9%), lauric acid (6.4%), myristic acid (5.1%) and phytol acetate (4.9%). For samples from Aindraham, 87.2% of the total oil content was identified and the main components were vitispirane (22.5%), palmitic acid (15.5%), linoleic acid (13.5%) and phytol acetate (6.3%).

Several works upon fragrance composition of rose flowers have been ascribed and for *R. canina* petals, the continuous superheated water extraction (SWE) of floral oils revealed to be mainly constituted by benzaldehyde, benzyl alcohol, phenylethyl alcohol, tetrahydroionol, eicosane and 2,6,11-trimethyldodecane (Özel and Clifford, 2004).

Diverse localities were responsible for different quantitative chemical composition of essential oils. Vitispirane, a norisoprenoid resulting from degradation of carotenoids, dominated the essential oil of sample from Aindraham. Temperature, pH of soil or even bad storage conditions of plant material could be responsible for the formation of vitispirane, since C_{13}-norisoprenoid compounds can arise from direct degradation of carotenoids or via glycosylated intermediates. These intermediates can release their volatile aglycone during fermentation processes or after acid hydrolysis according to the results obtained by some authors in other plant materials (Winterhalet and Schreier, 1988; Mendes-Pinto, 2009).

Total phenolic and flavonoid (flavones and flavonols) content of samples

The phenol content of methanolic extracts of *R. canina* samples growing in different localities and expressed in gallic acid equivalents/mL (GAE/mL) is depicted in Table 2. As can be seen, the level of total phenols is more heterogeneous in those samples from Aindrahan since the concentrations ranged from 5.42 ± 0.10 mg/mL, at Guoirya, to 9.21 ± 0.10 mg/mL, at Ain Zena. Several

Table 1. Percentage composition of the essential oils isolated from *R. canina* leaves.

Components	RI	Feija	Aindraham
Benzaldehyde	927	t	t
α-Pinene	930	t	t
n-Decane	1000	t	t
Benzene acetaldehyde	1002	0.8	t
cis-Linalool oxide	1045	t	t
2-Methyl decane	1058	t	t
trans-Linalool oxide	1059	t	t
n-Nonanal	1073	1.9	2.1
Linalool	1074	1.9	2.1
α-Campholenal	1088	t	T
n-Undecane	1100		
trans-Pinocarveol	1106	t	t
trans-Verbenol	1114	t	t
2-*trans*, 6-*cis*-Nonadienal	1114	t	t
Pinocarvone	1121	t	t
2-*trans*-Nonen-1-al	1124	t	t
Borneol	1134	t	t
Terpinen-4-ol	1148		
Methyl salycilate	1159		
α-Terpineol	1159	0.5	t
Myrtenol	1168	t	t
n-Decanal	1180	0.2	t
trans-Carveol	1189	t	t
Citronelol	1207	t	t
Geraniol	1236	t	t
Vitispirane*	1250	9.1	22.5
n-Undecanal	1288	0.2	0.4
Decanoic acid (= capric acid)	1350	t	t
trans-β-Damascenone*	1356	0.5	0.9
α-Ylangene	1371	0.8	t
α-Ionone	1399		
α-Gurjunene	1400	t	t
trans-β-Caryophyllene	1414	0.4	t
Geranyl acetone	1434	t	t
α-Himachalene	1441	t	2.4
trans-β-Ionone	1456	t	t
γ-Himachalene	1468	0.4	1.0
ar-Curcumene	1475	0.4	1.0
Viridiflorene	1487	0.2	0.9
α-Dehydro-*ar*-himachalene	1494	2.2	1.2
α-*trans,trans*-Farnesene	1500	t	t
n-Pentadecane	1500		
γ-Dehydro-*ar*-himachalene	1508	2.6	1.0
α-Calacorene	1525	0.6	0.4
Hexenyl benzoate*	1533		
Presilphiperfol-1-ene*	1544	3.9	3.7
Dodecanoic acid (= lauric acid)	1551	6.4	t
Spathulenol	1551	3.4	3.4
β-Caryophyllene oxide	1561	3.4	3.4
Globulol	1566	t	t

Table 1. Continued.

Humulene epoxide*	1580	2.3	2.0
n-Hexadecane	1600		
Benzyl benzoate	1701		
Tetradecanoic acid (= myristic acid)	1723	5.1	3.5
Hexadecanoic acid (= palmitic acid)	1908	23.2	15.5
Phytol acetate	2047	4.9	6.3
Linoleic acid	2125	7.9	13.5
% of Identification		83.2	87.2

RI = Retention index relative to C_9-C_{21} n-alkanes on the DB-1 column; t = trace (<t5%).
*Based on mass spectra only.

Table 2. Total phenol, flavonoid contents and antioxidant activities of *R. canina* extracts.

Locality	Places	Total phenolics (mg GAE/mL)	Total flavonoids (mg RE/mL)	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)
Feija	Rad el Koura	7.24 ± 0.10c	0.41 ± 0.01de	0.0226 ± 0.0003f	0.0116 ± 0.0004cd
	Blaket Statir	7.77 ± 0.10d	0.11 ± 0.01a	0.0152 ± 0.0003de	0.0125 ± 0.0004d
	Station Forestiere	7.26 ± 0.10c	0.31 ± 0.01c	0.0156 ± 0.0003e	0.0124 ± 0.0004d
	Sraa	8.62 ± 0.10e	0.22 ± 0.01b	0.0141 ± 0.0003bcd	0.0083 ± 0.0004b
Aindraham	Ain Zena	9.21 ± 0.10f	0.44 ± 0.01e	0.0148 ± 0.0003cde	0.0104 ± 0.0004c
	Guoirya	5.42 ± 0.10a	0.40 ± 0.01de	0.0130 ± 0.0003ab	0.00744 ± 0.0004ab
	Bni Mtir	6.65 ± 0.10b	0.38 ± 0.01d	0.0139 ± 0.0003bc	0.00611 ± 0.0004a
	Hammem Bourguiba	8.07 ± 0.10d	0.44 ± 0.01e	0.0125 ± 0.0003a	0.0105 ± 0.0004c

Values represent mean ± standard deviation of three replicates.
Values followed by the same letter under the same row, are not significantly different ($p > 0.05$).

factors seemed to be responsible for such variability, including altitude, developmental stage and climate, among others. Beyond the importance of these factors on the level of phenols they are also important on the type of phenol formed (Mohamed and Gerasopoulos, 1996; Toberman et al., 2008).

The most common procedure to evaluate the total flavonoid content is a spectrophotometric assay, based on the formation of a complex between the aluminium ion, Al^{3+} and the carbonyl and hydroxyl groups of the flavonoid. However, some works have demonstrated that this procedure has different responses depending on the flavonoid structure (Popova et al., 2004). In this way, in the present work we prefer to refer the levels of flavones and flavonols, since these groups of flavonoids are sole that are able to absorb in the range 390 - 440 nm owing to the presence of a double bond in positions 2-3 in the flavonoid skeleton (Popova et al., 2004).

In contrast to the phenol amounts, the flavonoid content was more heterogeneous in samples from locations at Feija. In this place, the lowest concentration was observed in samples from Blaket Statir (0.11 ± 0.01 mg/mL) and the highest concentration was registered in those samples from Rad el Koura (0.41 ± 0.01 mg/mL). All samples collected at Aindraham possessed ≈0.4 mg/mL of flavonoids (flavonols and flavones). It is also noteworthy to refer to an absence of correlation between phenol and flavones and flavonoids.

Carotenoids

Carotenoid pigments are abundant in many fruits and vegetables. Epidemiological studies have suggested that dietary β-carotene and lycopene may inhibit certain types of cancer acting either as pro-vitamin A or as chain-breaking anti-oxidants and thus protecting cells and organisms against photo-oxidation. Carotenoids are able to quench singlet molecular oxygen as well as scavenge radical species preventing lipid peroxidation (Edge et al., 1997).

Hodisan et al. (1997) reported as major carotenoids in *R. canina* fruits, determined by HPLC, β-carotene, lycopene, β-chryptoxanthin, rubixanthin, zeaxanthin and lutein. In the present work and according to the conditions of the laboratory, the carotenoids identified in fruit samples were β-carotene and lycopene. Table 3 presents the amounts of these pigments in fruit samples of *R. canina*.

In both samples, β-carotene predominated; contrasting with the results reported by Hodisan et al. (1997), in which the percentage of lycopene was higher than that of

Table 3. Carotenoids content and antioxidant activity of essential oils and carotenoid extracts.

Locality	β-Carotene (mg/mL)	Lycopene (mg/mL)	ABTS IC_{50} (mg/mL) Essential oils	ABTS (%) Carotenoid extracts
Aindraham	0.268 ± 0.009	0.050 ± 0.005	0.2018 ± 0.0052	46.82 ± 5.37
Feija	0.217 ± 0.029	0.031 ± 0.001	0.1590 ± 0.0040	28.42 ± 5.26

β-carotene. Samples belonging to Aindraham possessed higher amounts of both β-carotene (0.268 ± 0.009mg/mL) and lycopene (0.050 ± 0.005 mg/mL) than those growing in Feija (0.217 ± 0.029 and 0.031 ± 0.001 mg/mL, respectively). Diverse factors can be responsible for this quantitative variability of pigments, one of them being exposure to sunlight, since some authors reported an increase of carotenoids with high solar radiation, carotenoids could act as a safety valve venting the excessive visible radiation energy before it can damage the photosynthetic system (González et al., 2007).

Free radical scavenging activity of samples

Leaf extracts

The antioxidant activity determined in methanolic extracts of *R. canina* leaves showed that samples from those collected at Aindraham and Bni Mtir possessed the best capacity for scavenging the free radicals DPPH (2,2'-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), respectively (Table 2). No correlation was observed between the amounts of phenols or flavonoids in samples and the antioxidant activity measured by those two methods. These results are contradictory to those obtained by some authors for the same plant (Gao et al., 2000; Wenzig et al., 2008). However, it is important to state that different phenols possess diverse activity depending on their chemical structure. Phenolic compounds comprise one of the largest groups of plant metabolites and they can be divided into at least 10 different classes based on their general chemical structure: stilbenes, lignans, phenolic acids, flavonols, flavanols, isoglavones, flavanones, anthocyanidins, ellagitannis and proanthocyanidins (Scalbert and Williamson, 2000; Kondratyuk and Pezzuto, 2004). The capacity for scavenging free radicals from these classes of compounds differs and therefore, can partly explain the absence of correlation between the levels of phenols and flavonoids and antioxidant activity. Diverse group of phenols can be present in the extracts responsible for the diversity of the antioxidant activities detected.

According to Parejo et al. (2002), several reasons can be pointed out for these results: (a) extracts are very complex mixtures in which antioxidant and pro-oxidants can be present and where synergistic actions among compounds in the extract can occur, (b) the different methods of antioxidant activity evaluation are based on different mechanisms of reaction therefore, they can give different results and (c) antioxidant properties of single compounds within a group can vary remarkably. Therefore, the same levels of phenolic compounds do not necessarily correspond to the same antioxidant responses.

Essential oils

The antioxidant activity of leaf essential oils of *R. canina* is depicted in Table 3. Samples from Feija and Aindraham possessed antioxidant activity. However, the capacity for scavenging ABTS cation radical was higher in those samples from Aindraham. Such property may be partly due to the relative higher percentage of vitispirane present in this essential oil. The presence of two conjugated double bonds along with an oxygen atom (Figure 1) may stabilize, by resonance, the structure of vitispirane after giving an electron to the ABTS cation radical.

In spite of this antioxidant activity of the essential oils, such was not as pronounced as that described for leaf extracts. In this case, the activity was about tenfold higher than in essential oils.

Due to the weak oil yield obtained from leaves of *R. canina*, leaves of all localities of each zone were mixed giving an enough sample for extracting the essential oil. For the same reason, only one method for the determination of antioxidant activity was performed.

Carotenoids

Both extracts present some antioxidant activity, nevertheless, insufficient to determine IC_{50}. Twenty five microliters (maximal volume that this assay allows to use) of pure extract was not able to scavenge 50% of ABTS radical cation. The percentages given in Table 3 refer, therefore, to the activity of 25 μL of sample without any previous dilution. Samples from Aindraham (46.82 ± 5.37%) had higher activity than those from Feija (28.42 ± 5.26%). Such difference may be partly attributed to the highest levels of lycopen and β-carotene found in samples from Aindraham.

Such results did not disagree with those which referred that carotenoids are antioxidants. Oxidation can be prevented by diverse ways. Carotenoids are predominantly potent quenchers of reactive oxygen species such as

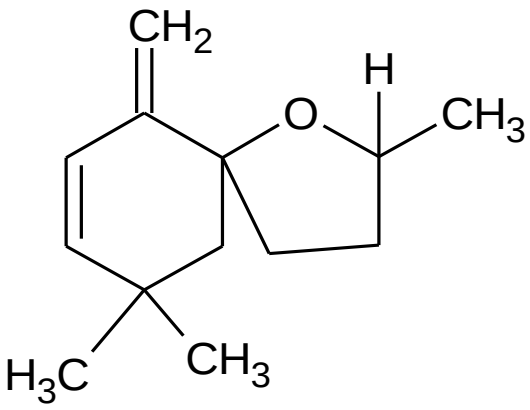

Figure 1. Chemical structure of vitispirane.

singlet molecular oxygen and peroxyl radicals, acting therefore as deactivators of excited molecules or as chain-breaking agents, respectively (Stahl et al., 1997). As well as the ability to quench excited states, carotenoids can also react with free radicals, nevertheless, depending on the oxygen concentration they can give rise to peroxyl radicals because during the reaction of a free radical with a carotenoid, carbon-centered radicals are formed, which react readily with oxygen originating those radicals (Edge et al., 1997).

The antioxidant activity determination of carotenoids through the method of DPPH was not performed due to the interference that occurs at the wavelength used to measure the depletion of the free radical DPPH (Jiménez-Escrig et al., 2000).

Conclusion

(1) The essential oils of *R. canina* leaves were predominantly constituted by palmitic acid and vitispirane and their concentrations were dependent on the harvesting zone.
(2) The phenol and flavonoid contents of samples were heterogeneous, depending predominantly on the collection locality.
(3) Concerning carotenoids, β-carotene predominated in all samples in detriment of the lycopene.
(4) Methanol extracts of leaves presented the best antioxidant activity when compared to those of essential oils and carotenoid extracts.

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