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Full Length Research Paper

Antioxidant activity of some Morrocan hydrosols

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Essential oils are used in Cosmetic, Perfumery, Food and Pharmaceutical Industries as flavours and/or medicines. However, part of the essential oil components that remains in the distillation water (hydrosol or distillate water) has been less studied both in chemical and biological terms. This research concerns the antioxidant activity, measured through several methods, of *Lavandula officinalis* L., *Origanum majorana* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L., *Cinnamomum verum* J. Presl. and *Syzygium aromaticum* (L.) Merrill and Perry hydrosols. The ability of hydrosols to prevent oxidation was checked by two main methods: prevention of lipid peroxidation through the measurement of malonaldehyde produced after degradation of hydroperoxides; and ability for scavenging free radicals including hydroxyl and superoxide anion radicals. The *S. aromaticum* and *T. vulgaris* hydrosols, predominantly constituted by eugenol and carvacrol, respectively, were the most effective as antioxidants, except for scavenging superoxide anion radical. In this case, *L. officinalis* hydrosol in which linalool prevailed was a stronger antioxidant. The worst hydrosol as antioxidant was that of *S. officinalis*, independent on the method checked.

Key words: Antioxidant, hydrosol, Lamiaceae, Lauraceae, Myrtaceae.

INTRODUCTION

Hydrosol derives from the Latin “hydro” meaning “water” and “sol” for “solution”. In the world of aromatherapy, hydrosols are also known as hydrolates, hydrolats, floral waters, and plant waters. Hydrolate uses “hydro” for “water” and “late”, from the French “lait”, for “milk”. In all chemistry terms, there is no specific reference to a previous distillation process and can be applied to any aqueous solution (Catty, 2001). Nevertheless, they are generally associated to the distillation processes for obtaining essential oils from aromatic plants. During distillation, there is a release of essential oils along with water from the steam or hydrodistillation. During distillation, part of the essential oil components remain dissolved in the distillation water. Typically, the hydrosol includes some of the water-soluble components of the essential oil as well as water-soluble plant components. The major components are generally the same of those present in oxygenated fraction of corresponding essential oils (Price and Price, 2004).

The presence of some components of essential oils gives the hydrosol its scent. Hydrosols are, therefore, quite fragrant, strongly flavoured and have a pH of 4.5 to 5.5 (Schorr, 2004; Tannous et al., 2004; Paolini et al., 2008). Despite the definition reported above, there is no legal definition for hydrosol per se nor grades and standards proposed by industry and the scientific community, nor specifications by international standardization boards and internationally recognized associations such as, ISO or ANFOR (Tannous et al., 2004).

Owing to the biological and organoleptic properties of hydrosols, they have been used in food and cosmetic industries. According to some authors, they are also used in biological agriculture against mushrooms, mildew, and insects and for fertilization of soils (Paolini et al., 2008).

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Abbreviations: NBT, nitroblue tetrazolium; **ABTS,** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); **TBARS,** thiobarbituric acid reactive species.

Rose and orange hydrosols (rose and orange waters) are traditionally used in medicine for skin care and in food for the preparation of cakes and beverages, mainly in the Mediterranean area (Paolini et al., 2008).

Only very few works have focused on the chemical composition of hydrosols, as well as their biological properties (antifungal, antibacterial and antioxidant) (Paolini et al., 2008, Lis-Balchin et al., 2003; Sağdiç and Özcan, 2003; Boyraz and Özcan, 2005; Özcan et al., 2008; Ulusoy et al., 2009).

In the continuation of our work on searching for natural antioxidants, the main goal of the present survey was to evaluate the antioxidant ability of seven commercial hydrosols produced by Moroccan industry: five belong to the Lamiaceae family (*Lavandula officinalis* L., *Origanum majorana* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L.), one two belonging to the Lauraceae and Myrtaceae families (*Cinnamomum verum* J. Presl. and *Syzygium aromaticum* (L.), Merrill and Perry, respectively).

MATERIALS AND METHODS

Chemicals

2-Thiobarbituric acid, nitrotetrazolium blue chloride, trichloroacetic acid, 2-deoxyribose, Trolox, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and fluorescein were purchased from Sigma-Aldrich Chemie, Steinheim, Germany.

Phenazine methosulfate (PMS), nicotinamide adenine dinucleotide disodium salt hydrate (NADH), 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH) and ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) were purchased from Acros organics, New Jerzey, USA.

di-Potassium hydrogen phosphate anhydrous (K_2HPO_4) and hydrogen peroxide (H_2O_2) were purchased from Panreac Quimica, Montcada i Reixac, Barcelona, Spain.

Potassium dihydrogen phosphate (KH_2PO_4) was purchased from Riedel-de-Haën Laboratory chemicals, Germany. Potassium chloride (KCl) was purchased from Analar Normapur, Geldenaaksebaan, Leuven, Belgium. Butan-1-ol was purchased from Fisher Scientific UK Ltd, Loughborough, UK.

Preparation of hydrosols

Sample (aerial parts) of each plant (1 kg) and bark and leaves of *Syzygium aromaticum* were alembic for steam distillation. Hydrosols of each plant (2 L) were obtained after 2 h. Then, the oil was removed by separation funnels. Hydrosols were kept in sterile bottles under refrigerated conditions until use. Samples of hydrosols obtained after distillation were diluted in distilled water for further analysis.

Chemical analysis of the essential oils

Chemical analysis of the essential oils gas chromatography (GC)

Gas chromatographic analyses were performed using a Autosystem XL (Perkin Elmer, Shelton, CT, USA) gas chromatograph equipped with two flame ionization detectors (FIDs), a data-handling system and a vapourizing injector port, into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J and W Scientific, Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30 m × 0.25 mm i.d., film thickness 0.15 μm; J&W Scientific). Oven temperature was programmed from 45 to 175°C at 3°C/min, then at 15°C/min to 300°C, then held isothermal for 10 min; injector and detector temperatures were 280 and 300°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using the split sampling technique, ratio 1:50.

Gas chromatography–mass spectrometry (GC–MS)

The GC–MS unit consisted of an Autosystem XL (Perkin-Elmer) gas chromatograph, equipped with a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J and W Scientific) and interfaced with a Turbomass mass spectrometer (software v. 4.1, Perkin-Elmer). 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; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C_9–C_{15} *n*-alkane indices and GC–MS spectra from a home-made library, constructed based on analyses of reference oils, laboratory-synthesized components and commercially available standards. Only the main components which concentrations were superior to 10% were considered in the present work.

Antioxidant activities

Thiobarbituric acid reactive species (TBARS)

The ability of the hydrosols to inhibit malondialdehyde formation, and therefore lipid peroxidation, was determined by using a modified thiobarbituric acid reactive species (TBARS) assay. Egg yolk homogenates were used as a lipid-rich medium obtained as described elsewhere (Dorman et al., 2005). Briefly, 0.5 ml of 10% (w/v) homogenate and 0.1 ml of samples with diverse concentrations were added to a test tube and made up to 1 ml with distilled water. Then, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% (w/v) TBA in 1.1% (w/v) sodium dodecyl sulphate (SDS) were added. The resulting mixture was vortexed and heated at 95°C for 60 min. After cooling, at room temperature, 5 ml of butan-1-ol was added to each tube; the contents of the tubes were stirred and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm using a Shimadzu 160-UV spectrophotometer. All of the values were based on the percentage antioxidant index (AI%), whereby the control was completely peroxidized and each oil demonstrated a degree of change; the percentage inhibition was calculated using the formula (1- *T*/*C*) × 100, where *C* is the absorbance value of the fully oxidized control and *T* is the absorbance of the test sample. The antioxidant capacity was determined from three replicates. The percentage antioxidant index was plotted against the concentrations of samples and IC_{50} values were determined (concentration of hydrosol to prevent 50% of lipid oxidation).

Hydroxyl radical scavenging activity

The assay of $^{\bullet}$OH-scavenging activity was developed according to Chung et al. (1997) with small modifications. Briefly, the reaction mixture was prepared with 10 mM $FeSO_4.7H_2O$, 10 mM EDTA, 10 mM 2-deoxyribose, 0.1 M phosphate buffer and sample in a test

tube to give a total volume of 1.8 ml. Finally, 200 µl of H_2O_2 was added to the mixture, which was incubated at 37°C for 4 h. After that, 1 ml trichloroacetic acid (2.8%) and 1 ml thiobarbituric acid (1%) were added to the test tube, which was boiled for 10 min. After cooling, its absorbance was measured at 520 nm in a Shimadzu 160-UV spectrophotometer. The $^{\bullet}$OH-scavenging activity (%) was calculated using the following equation:

Inhibition = $[(A_0-A_1)/A_0] \times 100$ (%)

Where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample.

Tests were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against hydrosol concentrations.

Superoxide anion scavenging activity

Measurements of superoxide anion scavenging activity of samples were based on the method described by Soares (1996). Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by reduction of nitroblue tetrazolium (NBT). The superoxide anion was generated in 3 ml of phosphate buffer (19 mM, pH7.4), containing NBT (43 µM) solution, NADH (166 µM) solution and different concentrations of hydrosols. The reaction was started with the addition of PMS solution (2.7 µM) to the mixture. The reaction mixture was incubated at 20°C for 7 min and the absorbance reading was performed at 560 nm in a UV/visible spectrophotometer, Ultrospec 1100 pro. The percentage of inhibition was calculated using the following equation:

Inhibition = $[(A_0-A_1)/A_0] \times 100$ (%)

Where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample. Tests were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against hydrosol concentrations.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) free radical-scavenging activity

The determination of ABTS radical scavenging was carried out as reported by Re et al. (1999). Briefly, the ABTS radical was generated by the reaction of (7 mM) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.7 at room temperature. Samples (10 ml) were added to 1490 ml ABTS, the absorbance at 734 nm was read at time 0 (A_0) and after 6 min (A_1). The percentage of inhibition was calculated using the following equation:

Inhibition = $[(A_0-A_1)/A_0] \times 100$ (%)

Where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample after 6 min. Tests were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against hydrosol concentrations.

Oxygen radical activity capacity (ORAC) assay

The ORAC method used, with fluorescein (FL) as the ‘‘fluorescent probe,” was that described by Ou et al. (2001). This assay is based on the capacity of antioxidants in a sample to quench peroxyl radicals that are generated from the thermal decomposition of AAPH (Sáenz et al., 2009). AAPH (0.414 g) was completely dissolved in 10 ml of 75 mM phosphate buffer (pH 7.4) to a final concentration of 153 mM and was kept in an ice bath. The unused AAPH solution was discarded within 8 h. Fluorescein stock solution (4.19×10^{-3} mM) was made in 75 mM phosphate buffer (pH 7.4) and was kept at 4°C in dark condition. The fluorescein stock solution at such condition can last several months. The (8.16×10^{-5} mM) fresh fluorescein working solution was made daily by further diluting the stock solution in 75 mM phosphate buffer (pH 7.4). Trolox standard was prepared as follows: 0.250 g of Trolox was dissolved in 50 ml of 75 mM phosphate buffer (pH 7.4) to give a 0.02 M stock solution. The stock solution was diluted with the same phosphate buffer to 50, 25, 12.5, and 6.25 µM working solutions. As the ORAC assay is extremely sensitive, the samples must be diluted appropriately before analysis to avoid interference.

In each well (96 well TC treated plate, black, µclear, Greiner Bio One) 150 µl of fluorescein working solution and 25 µl of sample, blank (75 mM phosphate buffer), or standard (Trolox) were placed. The plate was covered with a lid and incubated in the preheated (37°C) BioTek Synergy™ 4 Hybrid Microplate Reader for 10 min with a previous shaking of 3 min. AAPH was added to each well of the plate, except for the control and blank. The final volume of the assay was 200 µl. The microplate was shaken for 10 s, and fluorescence was read every minute for 90 min at excitation of 485 nm and emission of 527 nm.

ORAC values are calculated according to a previous paper (Cao and Prior, 1999). Briefly, the net area under the curve (AUC) of the standards and samples was calculated. The standard curve is obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values are calculated using the regression equation between Trolox concentration and the net AUC and are expressed as micromole Trolox equivalents per liter of samples.

Statistical analysis

Statistical comparisons were made with one-way ANOVA followed by Tukey multiple comparison test. The level of significance was set at $P<0.01$. Statistical calculation was performed using SPSS 15.0 software.

RESULTS AND DISCUSSION

In vitro antioxidant assays in foods and biological systems can be divided in two groups: those that evaluate lipid peroxidation; and those that measure free radical scavenging ability. For each group several methods may be found.

Generally, lipid substrates need accelerated oxidation conditions and temperature may be a way among others. Several tests are available for determining the primary (for example, hydroperoxides) and secondary products (for example, malonaldehyde) of lipid oxidation.

For measuring free radical scavenging ability, the methods are grouped in two sets, according to the chemical reactions involved: hydrogen atom transfer reaction-based methods and single electron transfer reaction-based methods.

In addition, tests evaluating effectiveness against several reactive oxygen species are also performed, such

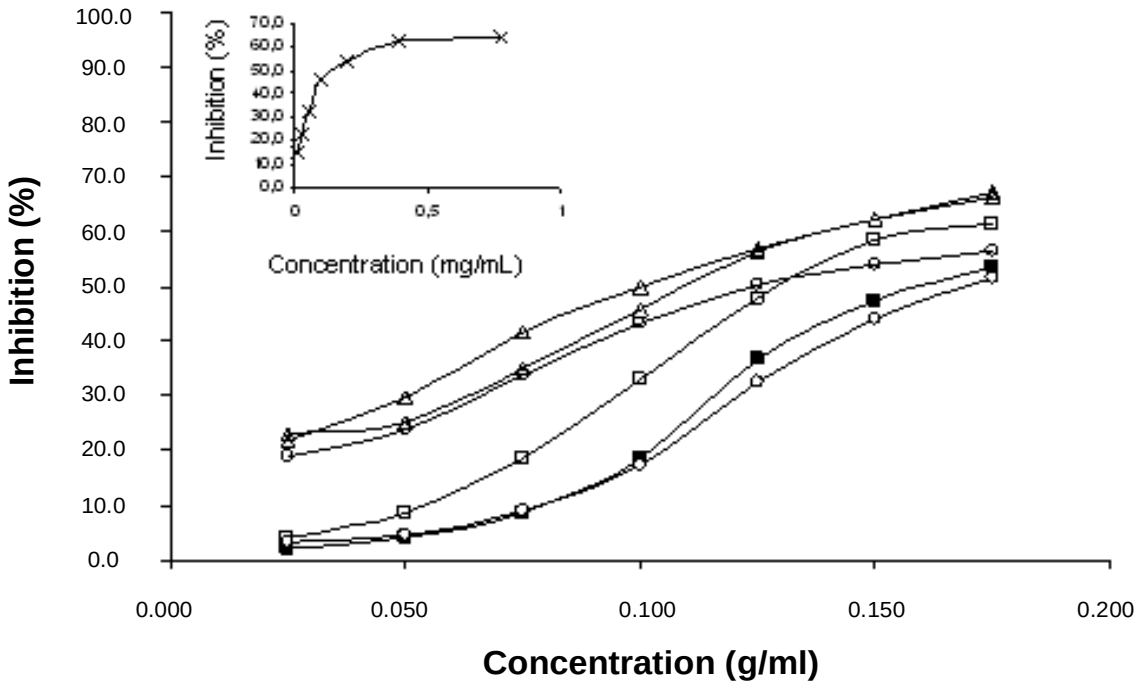

Figure 1. Percentage of inhibition of malonaldehyde formation, measured by the TBARS method, of hydrosols. (■) *Lavandula officinalis* L.; (•) *Origanum majorana* L.; (□) *Rosmarinus officinalis* L.; (o) *Salvia officinalis* L.; (▲) *Thymus vulgaris*; (△) *Cinnamomum verum* J. Presl.; (x) *Syzygium aromaticum* (L.) Merrill & Perry.

as assays for detecting the ability of samples for scavenging superoxide anion and hydroxyl radicals.

Based on their mode of action, the antioxidants can be classified as primary, secondary or co-antioxidants. Primary antioxidants are able to donate a hydrogen atom rapidly to a lipid radical, forming a new radical, which is more stable. Secondary antioxidants react with the initiating radicals (or inhibit the initiating enzymes), or reduce the oxygen level (without generating reactive radical species). Therefore, these secondary antioxidants can retard the rate of radical initiation reaction by elimination of initiators. This can be performed by deactivating high energy species (singlet oxygen); absorbing UV light; scavenging of oxygen; chelating metal that catalyses free radical reaction, or inhibiting enzymes, such as peroxidases, NADPH oxidase, xanthine oxidase, among other oxidative enzymes (Miguel, 2010). Therefore there is not an universal antioxidant by which at least two tests must be performed for detecting the possible antioxidant ability of one sample.

In the present work, lipid peroxidation was followed through the method known as TBARS, which measures the malonaldehyde, a secondary product of lipid oxidation using egg yolk as lipidic substrate, being temperature the chosen condition for accelerating the peroxidation process. One hydrogen atom transfer reaction-based method (ORAC) and one single electron transfer reaction-based method (ABTS) were used for evaluating the capacity of hydrosols for scavenging free radicals. More two methods were performed for checking the ability of hydrosols for scavenging superoxide anion and hydroxyl radicals. The capacity for preventing lipid peroxidation of hydrosols, evaluated by the TBARS method, is depicted in Figure 1. The best activity was found for *S. aromaticum* hydrosol, with significant (*P<0.01*) difference when compared to the remaining samples. At 0.5 mg/mL, the percentage of inhibition was 60%, not achieved by the remaining hydrosols. The antioxidant activity in all samples was concentration-dependent; nevertheless *S. aromaticum* hydrosol much more rapidly reached the saturation in contrast to the other hydrosols (Figure 1). The second best hydrosol as antioxidant was *T. vulgaris*. Regarding Table 1, in which the IC_{50} values are given, the best antioxidant activity is confirmed for *S. aromaticum* immediately followed by *T. vulgaris*. Nevertheless this hydrosol was about 500-fold inferior as antioxidant than *S. aromaticum*. The best activities found for these two hydrosols may be attributed to the presence of the main constituents of the respective oils, that is, generally the essential oils of those two species are mainly constituted by eugenol (*S. aromaticum*) (80.8%) and carvacrol (94.3%) (*T. vulgaris*) (Table 2). Despite the weak water solubility of these compounds, during the extraction process (steam distillation) of the essential oils, some amounts may have been arrested in water. Some authors (Ogata et al., 2000) also showed antioxidant activity of aqueous

Table 1. Antioxidant activity of hydrosols expressed as IC_{50} (mg/mL) for TBARS, ABTS, superoxide and hydroxyl; and as μM Trolox equivalent for ORAC method.

Plant	TBARS	ABTS	Superoxide	Hydroxyl	ORAC
L. officinalis	162.1±2.0^{b}	195.8±2.4^{c}	1.8±0.8^{d}	126.8±1.3^{d}	768.7±163.3^{cd}
O. majorana	123.6±2.0^{d}	74.5±2.4^{d}	9.3±0.8^{bc}	145.7±1.3^{c}	1003.4±163.3^{c}
R. officinalis	132.2±2.0^{c}	224.1±2.4^{b}	10.4±0.8^{ab}	98.5±1.3^{f}	690.6±163.3^{cd}
S. officinalis	170.0±2.0^{a}	313.4±2.4^{a}	-	193.4±1.3^{a}	282.6±163.3^{d}
T. vulgaris	101.6±2.0^{f}	3.0±2.4^{e}	6.3±0.8^{c}	154.3±1.3^{b}	3250.1±163.3^{b}
C. verum	110.4±2.0^{e}	217.5±2.4^{b}	11.0±0.8^{ab}	121.1±1.3^{e}	547.6±163.3^{cd}
S. aromaticum	0.2±2.0^{g}	1.1±2.4^{e}	12.9±0.8^{a}	58.3±1.3^{g}	14419.3±163.3^{a}

nd: not determined; P<0.01. Values in the same column followed by the same letter are not significantly different by Tukey multiple comparison test (P<0.01). Data are the mean of 3 replicates ± standard errors.

Table 2. Percentage of components present in the hydrosols.

Components	RI	*L. officinalis*	*O. majorana*	*R. Officinalis*	*S. officinalis*	*T. vulgaris*	*C. verum*	*S. aromaticum*
α-Pinene	930		2.4					
1-Octen-3-ol	961							t
p-Cymene	1003		2.4					
1,8-Cineole	1005	14.8		44.3	24.0	0.8		8.7
Limonene	1009		4.1					
Acetophenone	1017	t						
cis-Linalool oxide	1045	0.2						
Fenchone	1050							0.1
trans-Linalool oxide	1059	0.2						
α-Thujone	1073				3.6			0.1
Linalool	1074	45.0	1.5	2.6	0.1	0.3		0.1
β-Thujone	1081				12.9			0.1
trans-*p*-2-menthen-1-ol	1099		1.5					
Camphor	1102	15.7	0.3	11.6	51.0			6.7
cis-*p*-2-menthen-1-ol	1110		1.1					
trans-Verbenol	1114				0.3			
Borneol	1134	11.3	0.3	7.3	2.2	3.4		1.1
Terpinen-4-ol	1148		70.7		1.4	1.0		0.3
α-Terpineol	1159	11.8	9.0	5.0	2.2			0.3
Verbenone	1164			25.7				1.1
cis-Cinnamaldehyde	1169				1.9		t	
n-Dodecane	1200		t					
trans-Cinnamaldehyde	1224				0.1		91.9	
Thymol	1275					t		0.3
Carvacrol	1286					94.3		0.2
Eugenol	1327							80.8
% of Identification		99.0	93.3	96.5	99.7	99.8	91.9	99.9
Grouped components								
Monoterpene hydrocarbons			8.9					
Oxygen-containing monoterpenes		99.0	84.4	96.5	97.7	99.8		19.1
Phenylpropanoids					2.0		91.9	80.8
Others		t						t

RI: Retention Index relative to C_9-C_{15} *n*-alkanes on the DB-1 column; t: trace (<0.05%).

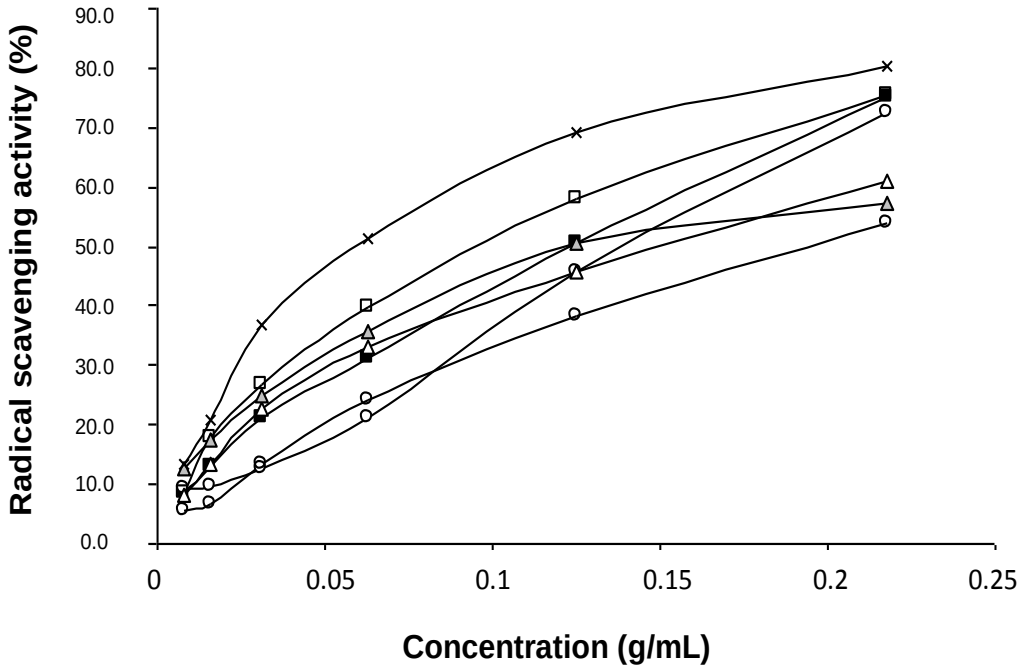

Figure 2. Percentage of hydroxyl radical scavenging of hydrosols. (■) *Lavandula officinalis* L.; (•) *Origanum majorana* L.; (□) *Rosmarinus officinalis* L.; (o) *Salvia officinalis* L.; (▲) *Thymus vulgaris*; (△) *Cinnamomum verum* J. Presl.; (•) *Syzygium aromaticum* (L.) Merrill and Perry.

extracts, despite the best activity found when organic solvents were used for extracting eugenol from clove.

The antioxidant activity of eugenol was already reported by Ogata et al. (2000). They suggested that eugenol may present an inhibitory effect on lipid peroxidation acting at the level of initiation. Jirovetz et al. (2006) found that the inhibitory lipid peroxidation of clove oil, mainly constituted by eugenol, was higher than the synthetic BHT (butylated hydroxytoluene). Also, using the TBARS method, such as in our work, other authors (Ruberto and Baratta, 2000) found that eugenol possessed better activity than carvacrol, mainly at high concentrations.

In addition to the capacity for preventing lipid peroxidation of eugenol, this phenylpropanoid was also able to scavenge hydroxyl and superoxide radicals (Ogata et al., 2000; Jirovetz et al., 2006). In our case, hydrosol of *S. aromaticum* was also capable for scavenging hydroxyl radicals, being even the best hydrosol for this purpose (Figure 2 and Table 1). The IC_{50} value was significantly inferior ($P<0.01$) to the remaining samples, due to the presence of eugenol. Nevertheless in this assay, *T. vulgaris* was not the second most important as scavenger of hydroxyl radicals. Dandlen et al. (2010) have also reported that thymol and/or carvacrol present in some *Thymus* species from Portugal did not present good ability for scavenging hydroxyl radicals, which according to the same authors such may reveal that these phenolic compounds are not determinant in the ability for scavenging these free radicals. Hydrosols of *R. officinalis* showed the second lowest IC_{50} value. In this case, 1,8-cineole and/or verbenone in *R. officinalis* seem to be more effective as hydroxyl scavengers than carvacrol. 1,8-Cineole, the main component of the *Salvia officinalis* oil, was also reported by Miguel et al. (2011) as good scavenger of hydroxyl radicals determined by the same method, mainly at lower concentration. In the present work, the hydrosol of *S. officinalis* also had relative high concentration of 1,8-cineole, but prevailing camphor. Such factor may contribute to the poorest hydroxyl scavenger activity of *S. officinalis* hydrosol (Figure 2, Table 2). Activities were also concentration-dependent as already reported in TBARS method. Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. With regard to the superoxide radical anion scavenging, clove hydrosol was not the best one. In this case, the hydrosol of *L. officinalis* was significantly better as superoxide scavenger than the remaining samples (Figure 3 and Table 1).

Linalool constituted the major components present in *L. officinalis* hydrosols (Table 2). This evident activity might be attributed to the relative high percentage detected of this monoterpenoid, in spite of Krishnakantha and Lokesh (1993) had considered that eugenol present in clover oil was better superoxide scavenger than linalool, in coriander oil. Nevertheless and for the first time, other studies demonstrated that unsaturated fatty acid

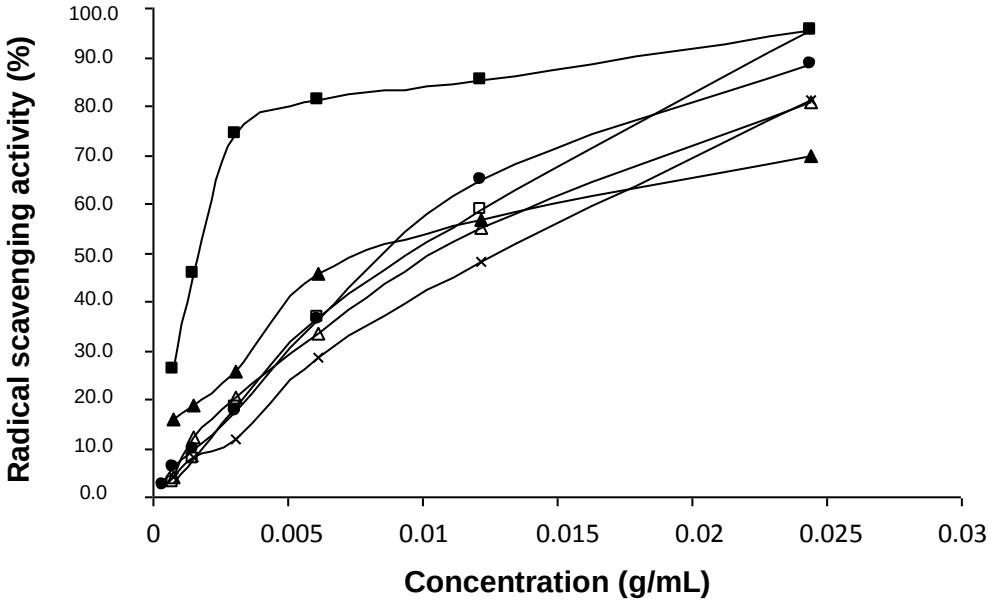

Figure 3. Percentage of superoxide radical scavenging of hydrosols. (■) *Lavandula officinalis* L.; (•) *Origanum majorana* L.; (□) *Rosmarinus officinalis* L.; (▲) *Thymus vulgaris*; (△) *Cinnamomum verum* J. Presl.; (x) *Syzygium aromaticum* (L.) Merrill and Perry.

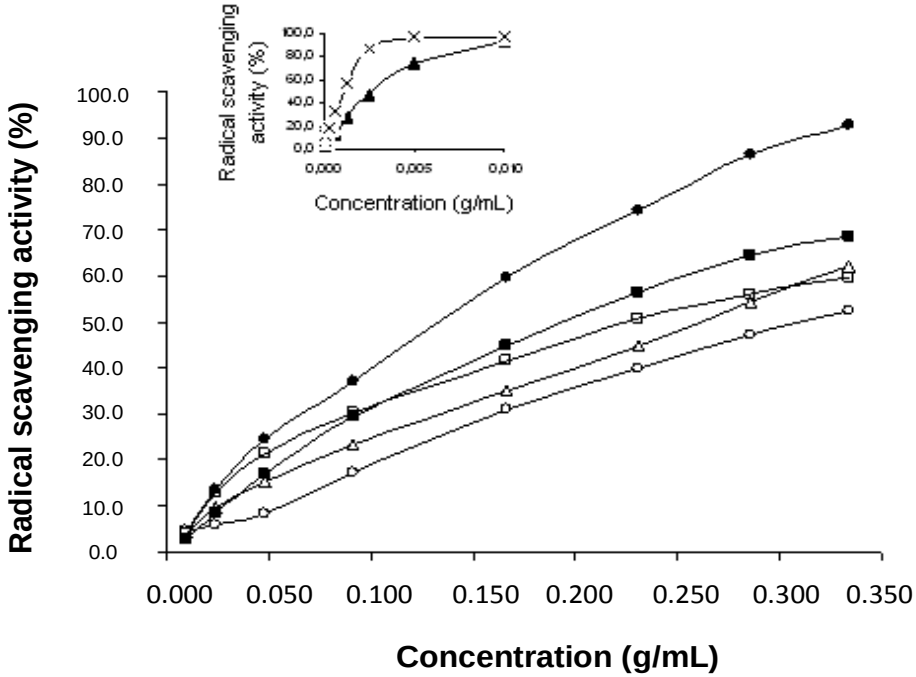

Figure 4. Percentage of ABTS radical scavenging of hydrosols. (■) *Lavandula officinalis* L.; (•) *Origanum majorana* L.; (□) *Rosmarinus officinalis* L.; (▲) *Thymus vulgaris*; (△) *Cinnamomum verum* J. Presl.; (x) *Syzygium aromaticum* (L.) Merrill and Perry.

peroxidation occurred in brain tissues of guinea pigs in H_2O_2-induced oxidative stress in the absence of linalool supplementation. However the authors did not report the possible mechanisms involved (Çelik and Özkaia, 2002). The hydrosol of *S. officinalis* was not evaluated in terms of capacity for scavenging superoxide radical anions. *S. aromaticum* and *T. vulgaris* hydrosols, mainly constituted by eugenol and carvacrol, respectively, were the most effective for scavenging ABTS free radicals (Figure 4 and Table 1). Such results were already expected since the

major components are phenolics with relative capacity for giving an electron and remaining stable by resonance. Comparing antioxidant activity of 30 plant species, some authors found that phenols, such as eugenol, possess relative good capacity for scavenging ABTS free radicals (Dudonné et al., 2009). According to other authors, such activity of eugenol can be attributed to the position of the single hydroxyl group on the phenol group (Hossain et al., 2008). Carvacrol and thymol were also reported as good scavengers of ABTS free radicals as expected due to their phenolic structure (Ündeğer et al., 2009; Damasceno et al. 2011). Therefore, eugenol and carvacrol can easily donate an electron to ABTS, remaining relatively stable, which means good antioxidant activity as free radical scavengers.

In ORAC method, a hydrogen atom transfer reaction-based assay, *S. aromaticum* followed by *T. vulgaris* revealed to be significantly more effective as chain breaking antioxidants than the remaining samples (Table 1). The poorest activity was found for *S. officinalis* hydrosol. These results confirmed those obtained when ABTS was used for measuring the antioxidant activity (Table 1), although diverse mechanisms are involved in the tests checked. Therefore, *S. aromaticum*, mainly constituted by eugenol may act as antioxidant by giving hydrogen or an electron. In both cases, the phenylpropanoid radical formed after the reaction is stable. Although *S. aromaticum* and *T. vulgaris* hydrosols possessed the best activities, a significant difference was detected between them, not found in TBARS method. Considering that eugenol and carvacrol, the most representative phenolic compounds present in the hydrosols, are responsible for the activities detected, carvacrol seems act preferentially as electron donor.

Conclusion

Many essential oils exhibit antioxidant activities. Phenols, such as carvacrol and eugenol constituting the major components of some of the essential oils, are among the most active natural antioxidants found in essential oils. Nevertheless, after obtaining the oils by steam distillation or hydrodistillation, part of the oxygenated components remained dissolved in the water. Generally this fraction, called hydrosol, is not used, although they are present. The present work confirmed the predominance of these oxygenated compounds as well as the importance of some of them as antioxidants. Eugenol and carvacrol prevailing in *S. aromaticum* and *T. vulgaris* hydrosols, respectively, were certainly responsible by the best capacity for scavenging free radicals demonstrated by the methods of ABTS, ORAC, and for preventing lipid peroxidation measured through the method of TBARS. Concerning scavenging of superoxide anion radicals, *L. officinalis* revealed to be the most effective, being linalool and the major component present in the hydrosol. *S. aromaticum* hydrosol was also the most active as scavenger of hydroxyl radicals.

In contrast, *S. officinalis* hydrosol had the poorest antioxidant activity in all assays checked. Therefore, industry can take advantage isolating both essential oils and hydrosols. Hydrosols have the benefit to be less aromatic and therefore more pleasant as scent but with recognized biological activities as verified in the present work.

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