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ORIGINAL PAPER

Antioxidant Activity and Chemical Content of Methanol and Ethanol Extracts from Leaves of Rockrose (*Cistus ladaniferus*)

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Introduction

Plants have been used for years as a source of traditional medicine to treat various diseases and conditions. Many of these medicinal plants are also excellent sources for phytochemicals, many of which have potent antioxidant activities [1, 2]. *Cistus ladaniferus* L., a *Cistaceae* named rockrose and the most important *Cistus* species in the perfumery field, is an odorous shrub that grows wild in Portugal, Spain, France (the Esterel massif), Italy (Sicily), and northern regions of Algeria and Morocco [3, 4]. This plant is widely used in herbal medicine and it is claimed to possess various physiological effects. Among the bioactive compounds present in plants, polyphenols and particularly flavonoids are widely appreciated for their potential beneficial health effects, like antioxidant, antimicrobial and anticarcinogenic activities [4]. Pharmacological studies on *Cistus* extracts reported that they have antibacterial, antifungal, anti-inflammatory [5], antiulcer [6], anti-aggregant of platelets [7], hypotensive and spasmolytic activities [8]. It is also commonly used as an antigastric agent by the local population of the North Morocco.

This specie is a strongly aromatic plant because of the high essential oil content in their leaves, flower, and fruit glands. The essential oil of *Cistus ladaniferus* is extremely complex. In some cases, up to 300 compounds have been detected by GC (gas chromatography), most of them being only present as traces [9]. The GC chromatogram of the oil from *C. ladaniferus* var. maculatus from Morocco showed more than 50 components among which 30 were identified. The main components were the monoterpenoids (bornyl acetate (5.5 %) and pinocarveol (7.7 %)), and sesquiterpenoid alcohols (viridiflorol (7.2 %) and ledol (3.5 %)) [10]. Moreover, this plant is rich in flavonoids and phenolic compounds [4].

Lipid peroxidation (LPO) is a major cause of food deterioration, affecting colour, flavour, texture and nutritional value [11]. Antioxidants are of interest to the food industry, because they prevent rancidity [11, 12]. At the present time, the most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate and tert-butyl hydroquinone. However, concern over the safety of synthetic antioxidants has increased consumers' interest in natural antioxidants. Therefore, there has been great interest in finding natural

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antioxidants (plant extracts, lisozims, bacteriocins, chitosan, etc.) to replace the synthetic ones [11–13].

A great number of plants worldwide have proved to present a strong antioxidant activity and a powerful scavenger activity against free radicals [2, 14–19]. Plant antioxidants are composed of a broad variety of different substances like ascorbic acid and tocopherol, polyphenolic compounds, or terpenoids. The antioxidant activities of phenolics are mainly due to their redox properties that allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential [16, 19, 20].

The aim of the present study was to evaluate and compare the antioxidant activities of methanol and ethanol extracts of *Cistus ladaniferus*. The extracts were investigated by several methods established for *in vitro* test, such as 1,1-diphenyl-2-picryhydrazyl (DPPH) radical-scavenging assay, reducing power assay, thiobarbituric acid test (TBARS), 2,2 azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay and metal chelating assay. Because of the important roles of the total phenolics and total flavonoids as antioxidants, the amounts of total phenolics and total flavonoids in the extracts were also determined. Both extracts were also analyzed by gas chromatography/mass spectrometry analysis (GC/MS) to identify the main components.

Materials and Methods

Plant Materials

Cistus ladaniferus L. (*Cistaceae*) plant was collected from Chefchaouen region (NW of Morocco) during the vegetation period and transported to the Laboratory of Biology and Health in the Abdelmalek Essaadi University. Collected plant materials were immediately dried in an oven (Selecta, Barcelona, Spain) at 35 °C. The leaves of plants were separated from the steam, and ground in a grinder (Moulinex, France).

Preparation of Extracts

Dried powders of leaves from *C. ladaniferus* were extracted with different solvents (methanol and ethanol). For methanolic and ethanolic extractions, a 25 g aliquot of each dried sample was extracted using 100 ml of methanol and ethanol respectively, at room temperature for 7 days. Two extraction replicates of each solvent were prepared for each plant sample. The extracts were filtered and the solvents were eliminated using a rotary evaporator (Buchi Heating Bath B-490, Buchi Rotvapor R-200) to obtain a dry extract. The extracts were stored at −20 °C until use.

Determination of Total Phenolic Content

The concentration of total phenols in extracts was measured by UV spectrophotometry, based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was Folin–Ciocalteu reagent [15]. To 0.5 ml of diluted extract (100 µg dry extract/ml solvent), 2.5 ml of Folin–Ciocalteu reagent (10 fold diluted in water) was added and, afterwards (within a time interval from 0.5 to 8 min), 2 ml of Na_2CO_3 (75 g/l) was added. The sample was incubated for 5 min at 50 °C and then cooled. For a control sample, 0.5 ml of distilled water was used. The absorbance of the resulting blue-coloured solutions was measured at 760 nm. Quantitative measurements were performed, based on a standard calibration curve of gallic acid in methanol. The mean (±SD) results of triplicate analyses were expressed as gallic acid equivalents (GAE) in milligram per gram of dry-extract.

Determination of Total Flavonoid Content

Total flavonoid content was determined by using a method described by Sakanaka et al. [17]. Briefly, 0.25 ml of the extracts (0.625–5 mg/ml) or rutin standard solution (15–250 mg/ml) was mixed with 1.25 ml of distilled water in a test tube, followed by addition of 75 µl of a 5 % (*w*/*v*) sodium nitrite solution. After 6 min, 150 µl of a 10 % (*w*/*v*) aluminium chloride solution was added and the mixture was allowed to stand for a further 5 min before 0.5 ml of 1 M NaOH was added. The mixture was made up to 2.5 ml with distilled water and mixed well. The absorbance was measured immediately at 510 nm. The mean (±SD) results of triplicate analyses were expressed as mg of rutin equivalents per gram of dry extract (mg RE/g).

Free Radical Scavenging Activity

The free radical scavenging activity of the extracts of methanol and ethanol of *C. ladaniferus* leaves were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) [21]. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of plant extract and was allowed to stand at room temperature for 30 min, and then absorbance was read at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: % RSA = $[(A_{DPPH} - A_{Extr})/\ A_{DPPH}] \times 100$. Where A_{DPPH} is the absorbance value of the DPPH blank sample, and A_{Extr} is the absorbance value of the test solution. A_{Extr} was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank.

ABTS Radical Cation Decolorization Assay

The antioxidant capacity assay was carried out using the improved ABTS radical cation decolorization assay as described by Re et al. [22]. $ABTS^{+}$ radical cation was generated by oxidation of ABTS with potassium persulfate. ABTS was dissolved in deionized water to 7 mM concentration, and mixed with 2.45 mM potassium persulfate. The reaction mixture was left to stand at room temperature in the dark for 12–16 h before use. The $ABTS^{+}$ solution was diluted with ethanol to an absorbance of 0.700±0.020 at 734 nm at 30 °C. Then, 1 ml of diluted ABTS solution was mixed with 10 µl aliquots of plant extracts, and the absorbance at 734 nm was measured at 30 °C exactly 6 min after mixing. Trolox standard solutions (concentrations from 0 to 2.5 mM) in 80 % ethanol were prepared and assayed using the same conditions. Appropriate solvent blanks were run in each assay. The percent of inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of trolox for the standard reference data. Results were expressed in terms of trolox equivalent antioxidant capacity (TEAC, mM trolox equivalents per mg dry extract). All determinations were carried out in triplicate.

Reducing Power Assay

The reducing power of the prepared *C. ladaniferus* extracts was determined according to the method of Oyaizu [23]. Briefly, variable concentrations of the extract and the standard compound (BHT) in 1 ml of solvent were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of a potassium ferricyanide solution (1 % *w*/*v*). The mixture was incubated in a water bath at 50 °C for 20 min. Next, 2.5 ml of a trichloroacetic acid (TCA) solution (10 %, *w*/*v*) was added, and the mixture was then centrifuged at 3,000 *g* for 10 min. A 2.5 ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 0.5 ml of a ferric chloride solution (0.1 %, *w*/*v*), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture correlates with greater reducing power.

Metal Chelating Activity

Metal chelating activity was determined according to the method of Decker and Welch [24], with some modifications. Briefly, 0.5 ml of the plant extract was mixed with 0.05 ml of 2 mM $FeCl_2$ and 0.1 ml of 5 mM ferrozine. Total volume was diluted 2 ml of methanol. Then, the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula given below:

$$\text{Scavenging effect } (\%) = \left[\left(A_{control} - A_{sample}\right)/A_{control}\right] \times 100.$$

Where $A_{control}$ is the absorbance of the ferrozine-Fe^{2+} complex and A_{sample} is the absorbance of the test compound. The percent of inhibition of absorbance at 562 nm was calculated and plotted as a function of concentration of ethylene diamine tetra acetic acid (EDTA) for the standard reference data. The absorbance of the resulting solution was compared to that of the calibrated EDTA standard. Results were expressed in terms of EDTA equivalents (mM EDTA equivalents per gram of dry extract). All determinations were carried out in triplicate.

Thiobarbituric Reactive Substances (TBARS) Test

The method of Kuppusamy et al. [12] was modified, to determine the conditions which would induce a high amount of thiobarbituric acid reactive substances (TBARS). The reaction mixture for inducing lipid peroxidation contained 1 ml fowl egg yolk emulsified with 0.1 M phosphate buffer (pH 7.4), to obtain a final concentration of 25 g/l and 100 µl of 1,000 µM Fe^{2+}. *Cistus* extracts or BHT were added to the incubation mixture to test the capacity to block lipid peroxidation under these conditions. Each assay was carried out in triplicate. The mixture was incubated at 37 °C for 1 h, afterwards it was treated with 0.5 ml of freshly prepared 15 % TCA and 1.0 ml of 1 % TBA. The reaction tubes were kept in a boiling water bath for 10 min. Upon cooling, the tubes were centrifuged at 3,500 *g* for 10 min to remove precipitated protein. The formation of TBARS was measured by removing 100 µl of the supernatant and measuring its absorbance at 532 nm.

Buffered egg with Fe^{2+} was used as control. BHT was used as the standard. The inhibition ratio was calculated

$$\%\,\text{inhibition} = \left[(A_0 - A_S)/A_0\right] \times 100$$

where A_0 refers to the absorbance of the control and A_s is the absorbance of the sample.

To determine the concentration required to achieve 50 % (EC_{50}) inhibition of phospholipid oxidation in egg yolk, the percentage of lipid peroxidation inhibition was plotted against extract concentration.

Chemical Composition

Gas Chromatography Analysis (GC)

The extracts were analyzed using a Shimadzu GC-17A equipped with flame ionization detector (FID) and HP-5

MS capillary column (30 m×0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 250 and 270 °C, respectively. Oven temperature was kept at 50 °C for 3 min, then gradually raised to 240 °C at 3 °C/min. Helium was the carrier gas, at a flow rate of 0.8 ml/min. Diluted samples (1/3 acetone, *v*/*v*) of 0.2 μl were injected manually in the split mode (split ratio 1/44). Quantitative data were obtained electronically from FID area data without using correction factors. All the tests were performed in triplicate.

Gas Chromatography/Mass Spectrometry Analysis (GC/MS)

Analysis of the extract was performed using a Shimadzu GC-17A equipped with a Shimadzu GCMSQP5050A mass selective detector and a HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium at a flow rate of 0.8 ml/min was used as carrier gas. Injector and MS transfer line temperatures were set at 250 and 270 °C, respectively. Oven program temperatures were the same as for the GC analysis. Diluted samples (1/3 acetone, *v*/*v*) of 0.2 μl were injected automatically in the split mode (split ratio 1/44). The components were identified by comparing their relative retention times and mass spectra with those of standards (for the main components), Wiley 229 library data of the GC/MS system, Kovats index and literature data. All the tests were performed in triplicate.

Statistical Analysis

The statistical analysis was performed by one-way ANOVA analysis of variance followed by Duncan's test, and results were considered to be statistically significant with a 95 % confidence level ($P<0.05$).

Results and Discussion

Antioxidant Capacity of *C. ladaniferus* Extracts

DPPH radical scavenging activity The radical scavenging activities of the extracts of *C. ladaniferus* were estimated by comparing the percentage inhibition of formation of DPPH radicals by the extracts and those of trolox and ascorbic acid (Fig. 1). This figure shows that the DPPH scavenging activity in both extracts was concentration-dependent (increasing from 50 μg/ml to 250 μg/ml) and the methanol extract exhibited considerably higher ($P<0.05$) DPPH radical-scavenging activities than ethanol extracts at all concentrations assayed, except for the concentration of 100 μg/ml. Overall, the methanol and ethanol extracts of *C. ladaniferus* were able to inhibit the formation of DPPH radicals with a percentage inhibition of 87.72 % and 50.10 % respectively at the highest concentration. At this concentration, the DPPH radical scavenging capacity of the methanol extract of *C. ladaniferus* was almost similar to those of trolox and ascorbic acid. It can be noted that extracts from *C. ladaniferus* show average inhibitory values that give an idea of the interesting antioxidant activity of such extract; with a high potential to take advantage of and develop nutraceutical products or additives for the food industry. The DPPH free radical assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and it is sensitive enough to detect active ingredients at low concentrations [21, 25].

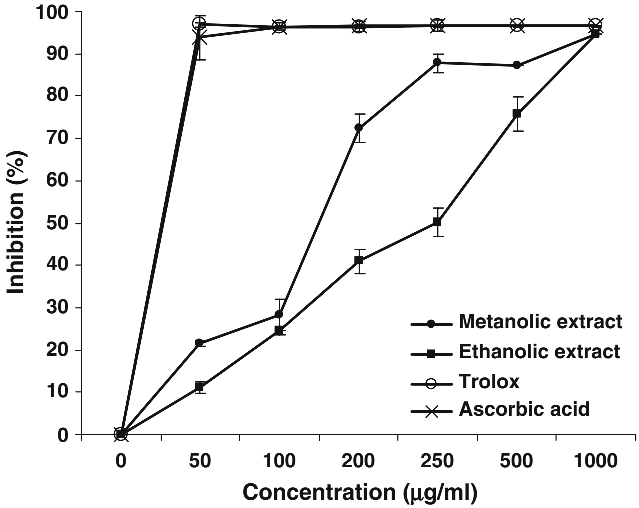


Fig. 1 Scavenging effects of leaves of *Cistus ladaniferus* methanol and ethanol extracts on DPPH radicals. Trolox and ascorbic acid were used as a positive control

ABTS radical scavenging activity To express the antioxidant capacity of methanol and ethanol extracts of *C. ladaniferus* by $ABTS^{\cdot+}$ scavenging assay, the TEAC (trolox equivalent antioxidant capacity) values after 6 min of reaction time were calculated and the results are shown in Table 1. Higher TEAC values demonstrate higher antiox-

Table 1 Radical-scavenging activities of *Cistus ladaniferus* extracts

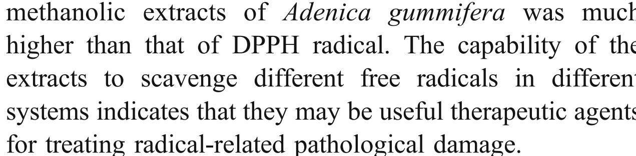
	ABTS[a] (TEAC mM Trolox/mg)	mM EDTA[a]/g extract
Methanol	1.26±0.01a	126.10±3.22a
Ethanol	0.73±0.01b	46.76±0.74b

[a] Values are means of triplicate determination

a–b: values within a column with different letters are significantly different ($P<0.05$)

idant activity. The methanol extract of *C. ladaniferus* exhibited the highest ($P<0.05$) radical scavenging activity when reacted with the $ABTS^{\cdot+}$ radicals. Figure 2 shows the percentage of inhibition on the absorbance of the $ABTS^{+}$ radical of methanol and ethanol extracts from leaves of *C. ladaniferus* at various concentrations (0−1,000 µg/ml). It was found that the inhibition percentage of the $ABTS^{\cdot+}$ radicals by the methanol extract increased linearly with concentration. The maximum inhibition was achieved at the maximum concentration assayed (1,000 µg/ml of the dry extract). On the contrary, the ethanol extract was considerably a less effective radical scavenger ($P<0.05$), showing a percentage of inhibition of 55.52 % at the highest concentration (1,000 µg/ml), almost similar to those of methanol extract (52.47 %) at a concentration of 250 µg/ml.

The DPPH radical scavenging activity of methanol extracts was higher ($P<0.05$) than that of $ABTS^{+}$ radical. At a concentration of 250 µg/ml, methanol extracts showed 87.72 % of DPPH radical inhibition in contrast with the 52.47 % of ABTS radical inhibition. The difference in the behavior of the extracts in the two radical scavenging assays may be explained by the different chemical mechanisms involved in the tests and the different chemical properties of the radicals. In other words, the radical system used for the antioxidant evaluation has some influences on the testing results; for example, the stoichiometry of reactions between the antioxidant compounds in the extract and $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ is different and the electron reduction potential of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ with respect to the compounds in the extracts are different. Also, stereoselectivity of the radicals may affect the capacity of the extracts to react with the radicals [26]. Adedapo et al. [27] reported that the scavenging of the $ABTS^{+}$ radical by the


methanolic extracts of *Adenica gummifera* was much higher than that of DPPH radical. The capability of the extracts to scavenge different free radicals in different systems indicates that they may be useful therapeutic agents for treating radical-related pathological damage.

Reducing power Figure 3 shows the concentration response curves for the reducing power of the methanol and ethanol extracts of *C. ladaniferus*. High absorbance indicates high reducing power. In this figure it can be observed that methanol extracts exhibited higher activity ($P<0.05$) than ethanol extracts at all concentrations assayed. The reducing power of methanol extract increased from 0.10 at 50 µg/ml to 1.47 at 1,000 µg/ml. These values were significantly higher ($P<0.05$) than those of the ethanol extract (0.02 at 50 µg/ml and 0.4 at 1,000 µg/ml). However, the reducing power of BHT was relatively more pronounced than that of the test samples. The reducing properties are generally associated with the presence of reductones, which is believed to break radical chains by donation of a hydrogen atom [28], indicating that the antioxidative properties are concomitant with the development of the reducing power. Therefore, the marked antioxidative activity of the methanol extract may be associated with its higher reducing power. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [18].

Ferrous ion-chelating ability The chelating effects of the test samples on ferrous ions are shown in Fig. 4. Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. Ferrous ions participate in direct and indirect initiation of lipid oxidation [29]. It was noted that the chelating ability of extracts increased ($P<0.05$) as a function of concentration. Methanol and ethanol extracts chelated ferrous ions by

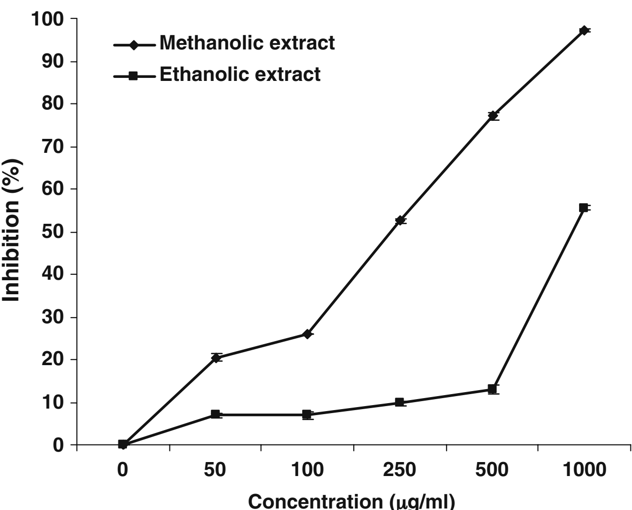


Fig. 2 $ABTS^{+}$ radical scavenging capacity of *Cistus ladaniferus* extracts

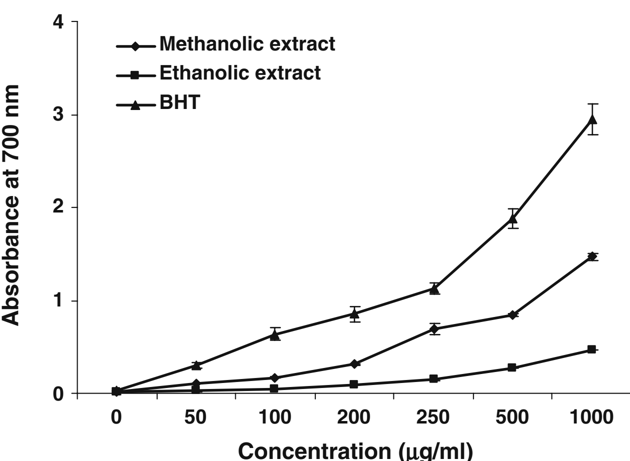


Fig. 3 Reducing power of *Cistus ladaniferus* extracts. BHT was used as a positive control

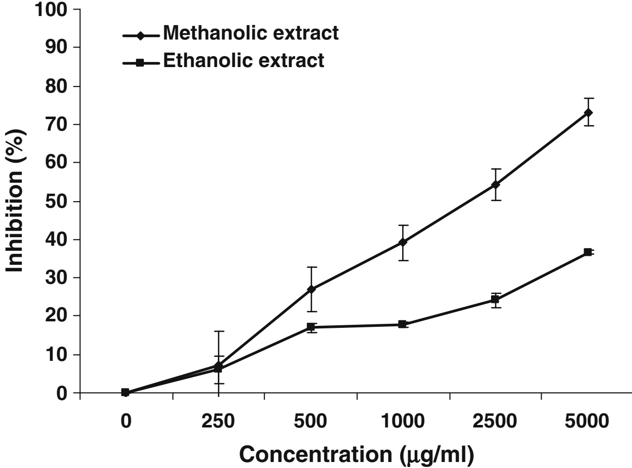


Fig. 4 Metal chelating ability of *Cistus ladaniferus* extracts

7.11 % and 6.04 % at 250 µg/ml, respectively whereas, at 5,000 µg/ml, the methanol extract showed an excellent chelating ability of 73.15 %, higher than that of the ethanol extract (36.68 %) ($P<0.05$). The ferrous ion-chelating ability of the extracts calculated as EDTA equivalents/g extract, is shown in Table 1. In this table it can be observed that the metal chelating activity of methanol was significantly higher ($P<0.05$; approximately 3 folds higher) than that of the ethanol extract. The iron (II) chelation properties of the additives may be attributed to their endogenous chelating agents, mainly phenolics [29]. Some authors have reported the antioxidant activity through the chelation of metal ions of some phenolic compounds [20].

Inhibition of lipid peroxidation of buffered egg The inhibitory effect of methanol and ethanol extracts from *C. ladaniferus* on TBARS production in egg yolk induced by Fe^{2+} is shown in Fig. 5. The percentage of lipid peroxidation inhibition values of methanolic extracts of *C. ladaniferus* were always higher ($P<0.05$) than those of ethanolic extracts, except for the highest concentration assayed, in this case both values of peroxidation inhibition were comparable ($P>0.05$). When ethanolic extracts were used at the lowest concentration, the lipid peroxidation inhibition was marginal (<10 %) but significant inhibitory response was evident as the concentrations were increased, reaching at 5,000 µg/ml an inhibition percentage higher than 60 %. The effect of antioxidant concentration on autoxidation rates depended on the antioxidant structure, nature of sample being oxidized and the oxidation conditions [16]. For all concentrations assayed, the positive control (BHT) showed higher ($P<0.05$) inhibition percentages than the extracts, ranging between 83.59 % at 100 µg/ml and 93.05 % at 5,000 µg/ml. On the other hand, the methanolic extract exhibited a better antioxidant action (EC_{50} value of 0.5 mg/ml) than the ethanol extract (EC_{50}<1 mg/ml) in the lipid peroxidation of buffered egg yolk. Lipid peroxidation can lead to the development of unpleasant rancid or off flavours as well as nutritional losses and formation of potentially toxic end-products [15], which is one of the major reasons for deterioration of foods during its processing and storage. Therefore, antioxidant compounds can scavenge free radicals and increase shelf life by retarding lipid peroxidation. In this study, the potencial of the methanolic extract at low concentrations suggests a possibility to use the extract in the food industry to make more appealing food and to increase shelf life by inhibiting oxidation.

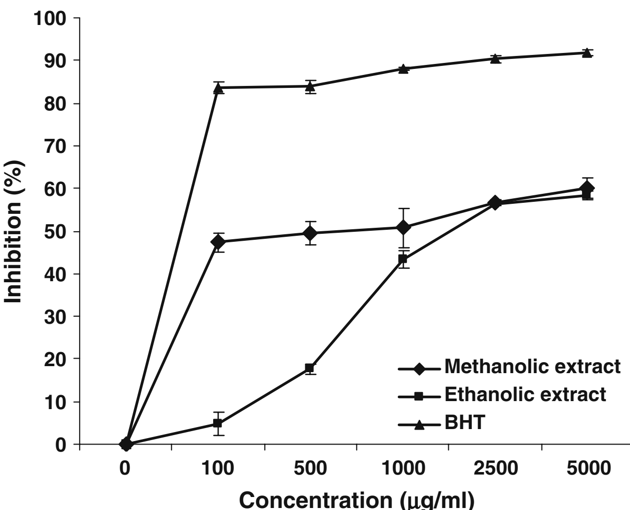


Fig. 5 Ability to inhibit lipid peroxidation of *Cistus ladaniferus* extracts

Determination of Total Phenolic and Flavonoid Content

As one of the most important antioxidant plant components, phenolic compounds are widely investigated in many medicinal plants and vegetables [2, 15]. The amount of total phenolic compounds and total flavonoids in the methanol and ethanol extracts of leaves of *C. ladaniferus* are shown in Table 2. The methanol extracts of *C.*

Table 2 The content of phenolic and the content of flavonoids in different extracts of leaves from *Cistus ladaniferus*

Plant extracts	Total phenolic[a] (mg GAE/g of extract)	Total flavonoids[a] (mg rutin/g of extract)
Methanol	18.43±2.74a	64.33±0.44a
Ethanol	11.87±0.53b	61.40±0.17b

[a] Values are means of triplicate determination

a-b: values within a column with different letters are significantly different ($P<0.05$)

Table 3 Constituents of methanol and ethanol extracts and their relative percentages of total chromatogram area and Kovats index

Pics	Compounds	KI	% Composition[a]	
			Methanol	Ethanol
1	Alpha thujene	923	0.73	0.25
2	Sabinene	970	0.80	0.59
3	α- terpinen	1,016	0.69	1.21
4	**Limonene**	**1,033**	**14.70**	**17.09**
5	Trans beta ocimene	1,058	2.07	3.22
6	Linalool	1,095	1.05	–
7	3-terpinenol	1,130	–	0.76
8	β-terpineol	1,161	–	1.72
9	Borneol	1,171	0.71	0.94
10	Terpinen-4-ol	1,180	2.50	2.13
11	Carveol	1,197	0.70	–
12	Bornyl acetate	1,285	0.69	–
13	Caryophyllene oxide	1,587	0.75	–
14	**Viridiflorol**	**1,599**	**38.38**	**40.76**
15	**Spathulenol**	**1,609**	**8.37**	**8.17**
16	Tetradecyl aldehyde	1,614	4.69	6.49
17	**Blumenol**	**1,710**	**23.17**	**16.67**

[a] Values are means of triplicate determination

ladaniferus exhibited the highest ($P<0.05$) total phenolic and flavonoid content. These differences can be explained by the different polarity of the solvents used; in this case, methanol extracted the most polar antioxidants. Similar results have been reported for other medicinal plants, in which solvents with different polarity were used. In all cases, the more polar was the solvent used, the higher the amount of total phenolic and flavonoid compounds were extracted [2, 15, 30]. In both extracts, the total flavonoid content was greater than the total phenolic content which has also been reported by several authors in different plant extracts [2, 31, 32]. Ciou et al. [31] suggested that hot air processing (during drying) might produce some compounds by Maillard browning or enzymatic browning, which could overestimate the total flavonoid content.

Chemical Composition of *C. ladaniferus* Extracts

Table 3 shows the main constituents found in the methanol and ethanol extracts from leaves of Moroccan *Cistus ladaniferus*. The components found were identified by comparing both their Kovats index and mass spectra with literature data and commercial mass spectra library. In the methanol extract were identified 15 components and only 13 in the ethanol extracts. In both extracts the major

Table 4 The major compounds of methanol and ethanol extracts of leaves from *Cistus ladaniferus*

Chemical structure	Name of compound	Chemical formulation
HO	Viridiflorol	$C_{15}H_{26}O$
OH, OH, O	Blumenol	$C_{13}H_{22}O_3$
	Limonene	$C_{10}H_{16}$
OH	Spathulenol	$C_{15}H_{24}O$

compounds identified were viridiflorol, blumenol, limonene and spathulenol. The content of the viridiflorol was 38.38 % and 40.76 % for methanol and ethanol extracts, respectively. The content of the blumenol was 23.17 % and 16.67 % for methanol and ethanol extracts, respectively. The content of the limonene was 14.70 % and 17.09 % for methanol and ethanol extracts, respectively. The content of the spathulenol was 8.37 % and 8.17 % for methanol and ethanol extracts, respectively. Some compounds were unique for methanol extract such as linalool (1.05 %), caryophyllene oxide (0.75 %), carveol (0.70 %) and bornyl acetate (0.69 %).

Some researchers reported that there is a relationship between the chemical structures of the most abundant compounds in the plants and their above mentioned functional properties [19]. From these results it can be concluded that the major components of *Cistus ladaniferus* extracts (viridiflorol, blumenol, limonene and spathulenol; Table 4) could have key roles for their functional properties. The antioxidant activity of limonene, veridiflorol, blumenol and spathulenol have been previously reported [33–35]. It is also possible that the minor compounds might be involved in some type of synergism with the other active compounds. Other than the major compounds, for example sabinene, terpinen-4-ol, borneol and α- terpinen as well as other minor constituents [35] of the extract of *Cistus ladaniferus* have also antioxidant activity.

With regard to the complex chemical composition of the methanol and ethanol extracts, it is quite interesting to note that some chemical compounds present in the methanol extract were not found in the ethanol extract. The compounds concerned included linalool and caryophyllen oxide (Table 3). This variation in chemical composition is however mirrored in the biological results, since the methanol extract exhibited higher activities in all of the assays. It has been reported that these compounds possess a strong antioxidant activity [36]. The presence of these compounds may have a synergistic effect or potentiating influence of this extract as observed in the antioxidant assays.

Conclusions

This study supports the idea that *Cistus ladaniferus* may be a good source of natural antioxidants to be used by the food industry. Methanolic extracts of *C. ladaniferus* exhibited the highest antioxidant activity in all the *in-vitro* assays applied (DPPH and ABTS radical scavenge activity, reducing power, metal chelating activity and inhibition of TBARS test), the highest level of total phenolic and flavonoid content. These extracts showed a high concentration of important compounds biologically active as viridiflorol, blumenol, limonene and spathulenol. However, further investigation to evaluate the practical effectiveness of the extracts in particular foods is needed.

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