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Antioxidant Activity and Total Phenolics Content of Montpellier Cistus (*Cistus monspeliensis*) Extracts

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Abstract

To determine the antioxidant activity of the water (WE), methanol (ME), ethanol (EE) and ethyl acetate extracts (EAE) from the leaves of *Cistus monspeliensis*, several methods such as 1,1-diphenyl-2-picryhydrazyl (DPPH) radical-scavenging assay, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay, reducing power assay, metal chelating assay, Thiobarbituric acid test (TBARS) and β-carotene linoleic acid system assay were investigated. Because of the important roles of total phenolics and total flavonoids as antioxidants, the amounts of total phenolics and total flavonoids in the extracts were also determined. The WE and ME showed a higher scavenging activity on the DPPH and ABTS radical. Both extracts also exhibited a strong chelating effect on Fe^{2+} and performed the best in the reducing power assay. On the other hand, the WE and ME were found to have the higher total phenolic contents (23.13 and 23.25 mg GAE/g of extract respectively) and total flavonoid contents (55.08 and 47.77 mg rutin/g of extract respectively). Thus, both extracts are promising alternatives to synthetic substances as food ingredients with antioxidant activity. The ME and the WE showed the similar levels in phenolic content.

Keywords

Cistus monspeliensis, Antioxidant Properties, Polyphenols, Natural Antioxidant

1. Introduction

Lipid peroxidation, or reaction of lipid with molecular oxygen, can be described generally as a process in which oxidants, such as free radicals, attack lipids containing carbon-carbon double bonds, particularly polyunsaturated fatty acids (PUFAs) [1]. The lipoperoxidation process has been implicated in several human diseases including atherosclerosis [2] [3], cancer [4], diabetes [5] and neurodegenerative disorders [6] [7]. Oxidation is also a significant contributor to a deteriorative reaction in food during storage and processing. Damage to cells in food products during storage can lead to serious health problems for consumers [8]. Consequently, to combat these effects, synthetic antioxidants are widely used in the food industry to neutralize free radicals that contribute to this deterioration. Common synthetic phenolic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG) [9]. However, these synthetic compounds have been linked to significant health concerns, including carcinogenicity, cytotoxicity, oxidative stress induction, and endocrine disruption [10] [11]. Therefore, the search for natural products with antioxidant activities has become a significant focus in food industry [12] [13]. Their effectiveness in combating stabilizing cellular functions under oxidative stress conditions, reducing inflammation, supporting skin health, and boosting immune function highlights their significance in nutrition [14] [15].

Many medicinal plants are excellent sources of phenolic compound which have been reported to exhibit potent antioxidant activity [16]-[19]. They have been demonstrated to have comparable activity to major biological antioxidants such as tocopherol and ascorbic acid [20]. Nevertheless, the potential antioxidant effect of phenolic compounds is directly dependent on the plant species used, the harvesting time, the growing conditions, and the type of the solvent used for extraction [21].

In the Mediterranean region, one of the most representative medicinal plants is *Cistus*. In Morocco, it is noted that *Cistus* seeds are consumed as appetizers and they are also possess aphrodisiac properties [22] [23]. In Spain, tea infusions are prepared for microbial infections [24]. Generally, *Cistus* compounds are particularly appreciated for their balsamic odour, as well as for their fixative properties [25]. The principal active constituents are polyphenolic compounds. Based on their chemical structures, these compounds are divided into various subclasses such as phenolic acid, flavonoids, tannins, anthocyanin [26]-[29]. The essential oil composition is chemically complex, revealing the presence of monoterpenes, sesquiterpenes hydrocarbon, oxygenated diterpenes, diterpenes hydrocarbon and oxygenated sesquiterpenes [30] [31].

One of the most common *Cistus* species in the Mediterranean basin is *Cistus monspeliensis* L. This medicinal plant is an aromatic shrub and is widespread in Morocco, except in desert areas [22]. The leaves are evergreen, elongated and highly aromatic, while the flowers boast five white petals and the fruits are small capsules. Extracts obtained from *C. monspeliensis* have been reported to possess

significant antimicrobial [29] [32] [33], antileishmanial [34], anti-inflammatory and analgesic [35], antiproliferative and cytotoxic activity [36]. Also, the significant inhibitory effects of *C. monspeliensis* extracts against the enzymes α-amylase and α-glucosidase demonstrate their potential abilities to reduce the postprandial increase of blood glucose levels in diabetic patients and their capacities to prevent type 2 diabetes [37]. However, these promising results have motivated further studies on this species, studying other biological activities that have not been previously considered.

The aim of the present study was to find total phenolic and flavonoid contents of water, methanol, ethanol and ethyl acetate extracts of Moroccan *C. monspeliensis*. Additionally, another goal of this study was to assess and compare the antioxidant activities of *C. monspeliensis* extract using different methods.

2. Materials and Methods

2.1. Chemical

1,1-diphenyl-2-picryl-hydrazil (DPPH) ferrozine, Folin-Ciocalteu reagent, gallic acid, rutin, aluminium chloride, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), potassium persulphate, ascorbic acid, butylated hydroxytoluene (BHT), iron (III) chloride, iron (II) chloride, trichloroacetic acid (TCA), Trolox, linoleic acid, tween 40 and β-carotene were from Sigma Chemical Company (Darmstadt, Germany). The solvent used for extraction, 2-thiobarbituric acid (TBA), sodium nitrite (II) and sodium carbonate were from Merck (Darmstadt, Germany). Potassium hexacyanoferrate was from Fluka BioChemika (GmbH Bosch, Germany).

2.2. Plant Materials

C. monspeliensis (*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. The taxonomic identification of plant material was confirmed by Dr. A. Ennabili (N˚ INP599 from The National Institute of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdellah University, Fés, Morocco). Collected plant materials were immediately dried in an oven (Selecta, Barcelona, Spain) at 35˚C for 3 days. The leaves of plants were separated from the steam, and ground in a grinder (Moulinex, France).

2.3. Preparation of Extracts

Dried powders of leaves from *C. monspeliensis* were extracted with different solvents (methanol, ethanol, ethyl acetate and water). For methanolic, ethanolic and ethyl acetate extractions, a 25 g of dried sample was extracted using 100 mL of methanol, ethanol and ethyl acetate respectively, at room temperature for 7 days. For aqueous extraction, a 25 g aliquot of dried sample was extracted using 100 mL of boiling water for 15 min. Two extraction replicates of each solvent were

prepared. The extracts were filtered from filter paper 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.

2.4. Determination of Total Phenolic Content (TPC)

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 [38]. To 0.5 mL of extract, 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 sodium carbonate (7.5% w/v) 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 mg of gallic acid equivalents (GAE) per gram of dry extract.

2.5. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined by using a method described by Sakanaka *et al.* [39]. Briefly, 0.25 mL of the extract 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 sodium hydroxide was added. The mixture was made up to 2.5 mL with distilled water and mixed. The absorbance was measured immediately at 510 nm. Quantitative measurements were performed, based on a standard calibration curve of rutin. The mean (±SD) results of triplicate analyses were expressed as mg of rutin equivalents (RE) per gram of dry extract.

2.6. Determination of Antioxidant Capacity

2.6.1. DPPH Radical Scavenging Capacity Assay

The free radical scavenging activity of the extracts of water, methanol, ethanol and ethyl acetate of *C. monspeliensis* leaves were measured by 1,1-diphenyl-2-picrylhydrazil (DPPH) [40]. 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 (50, 100, 200, 250, 500, and 1000 µg/mL) 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. Trolox and ascorbic acid were also used as controls. The inhibition percentage of the DPPH radical was calculated from the following equation: $\% I = [(A_{DPPH} - A_{Extr})/A_{DPPH}]*100$. Where A_{DPPH} is the absorbance value of the DPPH blank sample, and A_{Extr} is the absorbance value of the test solution. Each assay was carried out in triplicate.

2.6.2. 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.* [41]. $ABTS^+$ 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 extract at different concentrations (50, 100, 250, 500, and 1000 µg/mL), 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 inhibition percentage of the ABTS radical was calculated from the following equation: $\% I = [(A_{ABTS} - A_{Extr})/A_{ABTS}]*100$. Where A_{ABTS} is the absorbance value of the ABTS blank sample, and A_{Extr} is the absorbance value of the test solution. Where A_{ABTS} is the absorbance value of the ABTS blank sample, and A_{Extr} is the absorbance value of the test solution. Results were expressed also in terms of trolox equivalent antioxidant capacity (TEAC, mM trolox equivalents per mg dry extract). All determinations were carried out in triplicate.

2.6.3. Reducing Power Assay

The reducing power of the prepared *C. monspeliensis* extracts was determined according to the method of Oyaizu [42]. One mL of plant extracts at different concentrations (50, 100, 200, 250, 500, and 1000 µg/mL) 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 TCA solution (10%, w/v) was added, and the mixture was then centrifuged at 3000 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. BHT was also used as control. Each assay was carried out in triplicate.

2.6.4. Metal Chelating Assay

Metal chelating activity was determined according to the method of Decker and Welch [43] with some modifications. Briefly, 0.5 mL of the plant extract was mixed with 0.05 mL 2 mM $FeCl_2$ and 0.1 mL 5 mM ferrozine. Total volume was diluted with 2 mL 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 absorbance of the resulting solution was compared to that of the calibrated ethylene diamine tetra acetic acid (EDTA). Results were expressed in terms of EDTA equivalents (mM EDTA equivalents per gram of dry extract). All

determinations were carried out in triplicate.

2.6.5. Tiobarbituric Acid Reactive Species Test

The method of Kuppusamy *et al.* [44] 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 mM Fe^{2+}. *Cistus* extracts or BHT was added to the incubation mixture to test the capacity to block lipid peroxidation under these conditions. The mixture was incubated at 37˚C for 1 h, afterwards it was treated with 0.5 mL of freshly prepared TCA (15%) and 1 mL of TBA (1%). The reaction tubes were kept in a boiling water bath for 10 min. Upon cooling, the tubes were centrifuged at 3500 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 from the following equation:

$$\% \text{ inhibition} = ((A_{control} - A_{sample})/A_{control}) * 100$$

where $A_{control}$ refers to the absorbance of the control and A_{sample} is the absorbance of the sample. Each assay was carried out in triplicate.

2.6.6. β-Carotene Linoleic Acid System Assay

A stock solution of β-carotene linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 mL of chloroform, and 25 µL of linoleic acid and 200 mg of Tween 40 were added [45]. The chloroform was removed using a rotary vacuum evaporator. Then, 100 mL of distilled water was added to the residue with vigorous shaking to form an emulsion. A test sample (350 µL of the extracts, prepared at 2 g/L concentration) was mixed with 2.5 mL of this emulsion. The reaction mixture was incubated at room temperature for 48 h for auto-oxidation, and then the absorbance was measured at 490 mm. The same procedure was repeated with BHT as positive control. Relative antioxidant activities (RAA%) of the extracts were compared with those of BHT. The assays were carried out in triplicate.

2.7. Statistical Analysis

All data was reported as mean ± standard error of triplicate determinations and analyzed using one-way analysis of variance (ANOVA) with significant differences between means determined at $p < 0.05$.

3. Results and Discussion

3.1. Determination of Total Phenolic (TPC) and Total Flavonoid Content (TFC)

The total amount of phenolic content present in the water extract (WE), methanolic extract (ME), ethanolic extract (EE) and ethyl acetate extract (EAE) of *C.*

monspeliensis is shown in Table 1. The ME and the WE showed the similar levels (23.25 and 23.13 mg GAE/g of extract, respectively) in phenolic content ($p < 0.05$). The total phenolic content in EE was 21.44 mg GAE/g of extract, while the lowest content was measured for EAE (14.38 mg GAE/g of extract). The flavonoid content of the extracts is significantly different, the WE and ME are rich of flavonoids with a value of 55.08 and 47.77 mg rutin/g of extract respectively. However, the EAE yielded the lowest ($p < 0.05$) flavonoid content (23.56 mg rutin/g of extract) (Table 1). In our experiment, a considerable variability in phenolic and flavonoid contents was observed between extracts. Specifically, the polyphenol content is higher in WE and ME, and lower in EAE. The yield of phenolic compounds increased with increasing solvent polarity. These findings, congruous with previous studies demonstrating that the concentration of polyphenol compounds depends on the polarity of extraction solvents [46]-[50].

Table 1. The content of phenolic and the content of flavonoids in different extracts of leaves from *Cistus monspeliensis*.

Plant extracts	Total phenolic* (mg GAE/g of extract)	Total flavonoids* (mg rutin/g of extract)
Methanol	23.25 ± 0.17a	47.77 ± 5.79b
Ethanol	21.44 ± 0.97b	33.21 ± 1.15c
Ethyl acetate	14.38 ± 0.67c	23.56 ± 3.03d
Water	23.13 ± 0.29a	55.08 ± 3.07a

*Values are means of triplicate determination, a-d: values within a column with different letters are significantly different ($p < 0.05$).

Interestingly, the results confirmed the potential for recovering high amounts of phenolics content with antioxidant properties of different extracts of *C. monspeliensis*. Several reports highlight the contribution of phenolic compounds to the antioxidant potential of different plant extracts [51]-[53]. Phenolic compounds can play an important role in neutralizing and absorbing free radicals, efficiently inhibiting lipid peroxidation [54] [55]. For these reasons, the findings suggest that these extracts could be applied in various contexts, rich in polyphenolic compounds, to delaying, retarding, or preventing the chemical and enzymatic reactions associated with lipid oxidation.

3.2. DPPH Radical Scavenging Activity

The radical scavenging activities of the WE, ME, EE and EAE of *C. monspeliensis* leaves were estimated by comparing the percentage inhibition of DPPH radicals by the extracts and those of trolox and ascorbic acid (Figure 1).

The results indicated that the DPPH free radicals were scavenged by all plant extracts in a concentration dependent manner. At low concentrations (100 µg/mL), the antioxidant activity of WE (12.53%) was comparable to that of EE (12.62%) ($p < 0.05$). In contrast, the ME exhibited higher antioxidant activity

(45.66%). The antioxidant activity levels of EAE were very low, accounting for less than 2% of the total.

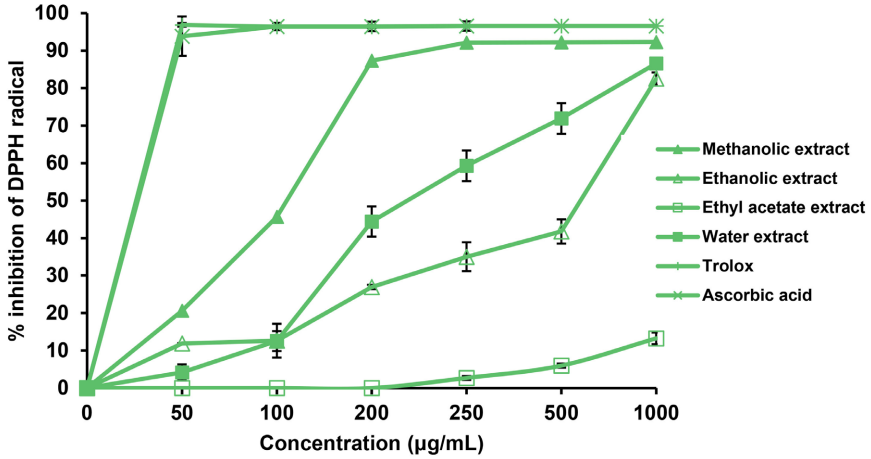

Figure 1. Scavenging effects of leaves of *Cistus monspeliensis* extracts on DPPH radicals. Trolox and ascorbic acid were used as a positive control.

At 200 µg/mL, the ME of *C. monspeliensis* demonstrated excellent DPPH scavenging activity (87.29%), comparable to that of synthetic antioxidant standard Trolox and ascorbic acid (96.39% and 96.47% respectively), followed by the WE and EE (44.41% and 26.96% respectively).

At 250 µg/mL, the ME and WE exhibited high ($p < 0.05$) scavenging activity with 92.20% and 71.90% respectively, significantly higher ($p < 0.05$) than EE (41.77%). An exception was observed in the EAE, which did not show a leveling off with increasing concentration (2.98%). However, the radical scavenging activity of this extract (EAE) was much lower than the other extracts ($p < 0.05$); even at higher concentration (1000 µg/mL), the scavenging activity was 13.25%. This observation is fairly common in plant extracts; many non-polar compounds are not able to act as antioxidants [56].

DPPH method has been used as one of basic screening steps for searching new antioxidant compounds in extracts from natural resources [57] [58]. Conventionally, high free radical scavenging ability is regarded as high antioxidant activity. In our study, the WE and ME exhibited the highest antioxidant activity. These variations in activity among the extracts can be explained by the contribution of compounds phenolic radical-scavenging activity which has been widely studied. Effectively, the WE and ME showed the highest phenolic and flavonoid contents, compared with EE and EAE.

3.3. ABTS Radical Scavenging Activity

The ABTS radical cation decolourisation test is another method widely used to assess antioxidant activity. Reduction in colour indicates reduction of ABTS radical [59].

Figure 2 shows the percentage of inhibition on the absorbance of the $ABTS^{+}$

radical of WE, ME, EE and EAE extracts from leaves of *C. monspeliensis* at various concentrations (50 - 1000 µg/mL). At low concentrations tested (50 - 100 µg/mL), the WE, ME and EE of *C. monspeliensis* exhibited very similar values (13.67% - 16.03%, 15.08% - 15.88% and 17.81% - 20.77% respectively). At dose of 500 µg/mL, the WE and ME were highly effective ($p < 0.05$) in free radical scavenging activity (52.47% and 67.69% respectively) compared to EE (31.94%). Increasing the concentration of ME and WE (1000 µg/mL) resulted in increased ($p < 0.05$) antioxidant activity to 90.13% and to 97.82% respectively, in contrast to the 62.30% of radical inhibition of EE. In this assay, the EAE showed insignificant activity; this extract reduced the ABTS radical by 34.4% at the highest dose of 1000 µg/mL ($p < 0.05$).

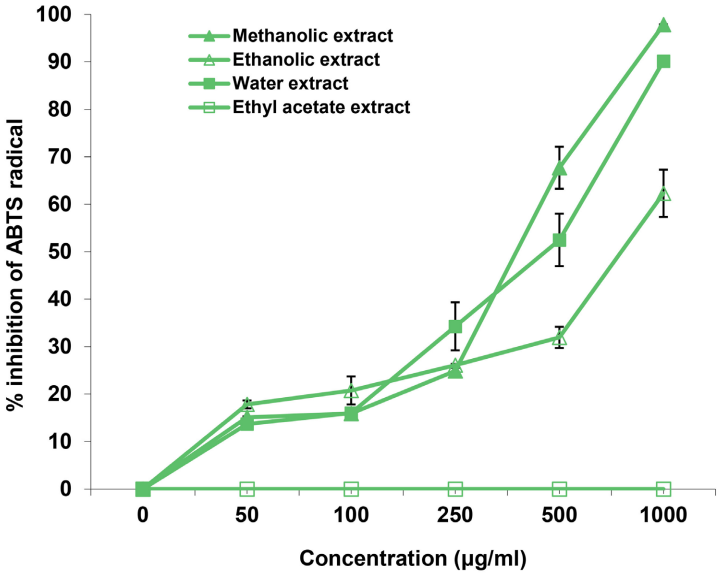

Figure 2. Scavenging effects of leaves of *Cistus monspeliensis* extracts on ABTS radical.

In this study, the ABTS· scavenging activity was expressed in terms of a TEAC (trolox equivalent antioxidant capacity) value after 6 minutes of reaction time, with a higher value indicating a more potent radical-scavenging effect. The results indicate that WE (1.30 mM Trolox/mg) and the ME (1.42 mM Trolox/mg) are a much more powerful antioxidants than that of the EE (0.88 mM Trolox/mg) ($p < 0.05$). This variation in the activity is related to some compounds that may not react as quickly as Trolox, resulting in low TEAC values for early measuring times [60].

3.4. Reducing Power Activity

In the reducing power assay, the presence of reductants in the tested samples would result in reducing Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). The Fe^{2+} can therefore be monitored by measuring the formation of Perls Prussian blue at 700 nm [61]. High absorbance indicates high reducing power. It can be

observed that the reducing power of the WE of *C. monspeliensis* was superior ($p < 0.05$) than the other extracts at 50 - 500 µg/mL (Figure 3). In fact, the reducing power of WE increased from 0.22 at 50 µg/mL to 1.31 at 500 µg/mL. However, the reducing power of ME increased from 0.12 at 50 µg/mL to 0.93 at 500 µg/mL, and that of EE and EAE increased from 0.04 at 50 µg/mL to 0.31 at 500 µg/mL and from 0.02 at 50 µg/mL to 0.06 at 500 µg/mL respectively. At a dose of 1000 µg/mL, the reducing power of WE and ME was the most effective ($p < 0.05$) (1.62 and 1.57 respectively) and significantly higher ($p < 0.05$) than that EE and EAE (0.65 and 0.13 respectively). However, the reducing power of BHT was relatively more pronounced ($p < 0.05$) than that of the test samples (from 0.30 at 50 µg/mL to 1.87 at 500 µg/mL).

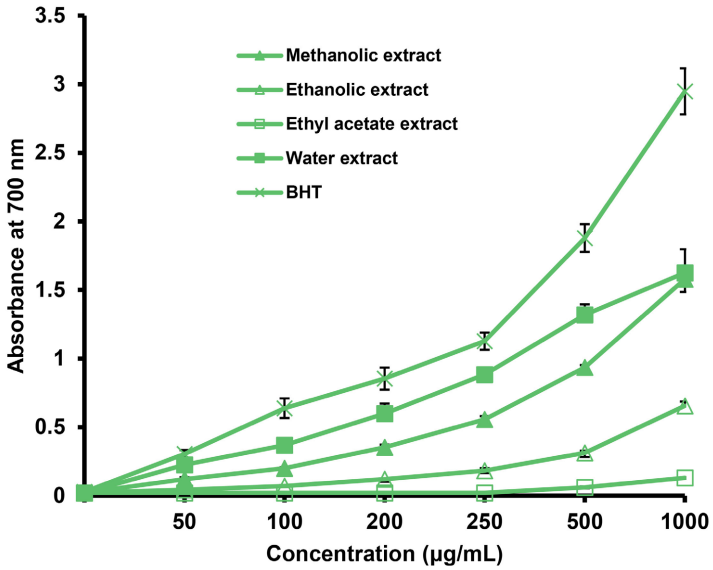

Figure 3. Reducing power of *Cistus monspeliensis* extracts. BHT was used as a positive control.

The reducing properties are generally associated with the presence of reductones [62] [63]. Therefore, the WE and ME of *C. monspeliensis* might contain reductones, which could react with free radicals to stabilize and terminate radical chain reactions. It has been previously reported that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [64] [65].

3.5. Ferrous Ion-Chelating Ability

Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of ion chelating agents, the complex formation is disrupted, resulting in a decrease in the red color of the complex. Table 2 presents the ferrous ion-chelating ability of the different extracts of *C. monspeliensis* calculated as EDTA equivalents/g extract. Apparently, the WE showed the highest ($p < 0.05$) ferrous iron chelating ability (130.54 mM ETDA/g extract). The second-highest abilities were observed in ME

(111.42 mM ETDA/g extract). However, the EE showed relatively lower ($p < 0.05$) chelating activity (82.21 mM ETDA/g extract) when compared to the action obtained from the WE and ME, but higher ($p < 0.05$) than EAE (8.81 mM ETDA/g extract).

Table 2. Radical-scavenging activities of *Cistus monspeliensis* extracts.

Plant extracts	ABTS* ($TEAC_{6min}$ mM Trolox/mg)	mM ETDA*/g extract
Methanol	1.42 ± 0.01a	111.42 ± 6.01b
Ethanol	0.88 ± 2.58c	82.21 ± 2.58c
Ethyl acetate	nd	8.81 ± 3.54d
Water	1.30 ± 0.29b	130.54 ± 7.29a

*Values are means of triplicate determination, a-d: values within a column with different letters are significantly different ($p < 0.05$), nd: Not determined.

The ferrous ion (Fe^{2+}) is currently recognized as the more pro-oxidative form of iron [66]. Minimizing ferrous (Fe^{2+}) ions may provide protection against oxidative damage by inhibiting the production of reactive oxygen species and lipid peroxidation. Metal ions could be inactivated and potentially inhibited by chelating agents, such as a phenolic compound [67]. This study demonstrates that the WE and ME of *C. monspeliensis* leaves have a marked capacity for iron binding, attributed to the presence of polyphenols with potent iron-chelating capacity.

3.6. Tiobarbituric Acid Reactive Species

Figure 4 shows the results for the TBARS values of WE, ME, EE and EAE from *C. monspeliensis*. At 100 µg/mL, the capacity to protect lipids from oxidation of all extracts showed a percentage of inhibition lower than 30%. However, at 500 - 5000 µg/mL ($p > 0.05$), WE exhibited a higher percentage of inhibition compared to the other extracts. The values ranged from 47.67% to 70.86% for WE, from 30.99% to 54.89% for ME and from 24.34% to 52.27% for EE. The result of this assay suggests that the antioxidants in the different extracts required a high concentration to have a significant activity. However, at all concentrations, BHT significantly ($p > 0.05$) reduced the TBARS values compared to the different extracts, with the values ranging between 83.59% at 100 µg/mL and 93.05% at 5000 µg/mL.

Thiobarbituric acid reactive substance (TBARS) assay is another method to detect lipid oxidation. This assay measures malondialdehyde (MDA), which is a split product of an endoperoxide of unsaturated fatty acids resulting from oxidation of lipid substrates [68]. The major strategies for preventing lipid oxidation involve the use of antioxidants. In the past few years, various plant extracts containing phenolic compounds have been demonstrated to be effective antioxidants in food [69]-[72]. Therefore, the antioxidant properties of WE in this assay, which has already shown a high concentration of phenolics, suggest possible alternatives to minimize or retard oxidative deterioration in food.

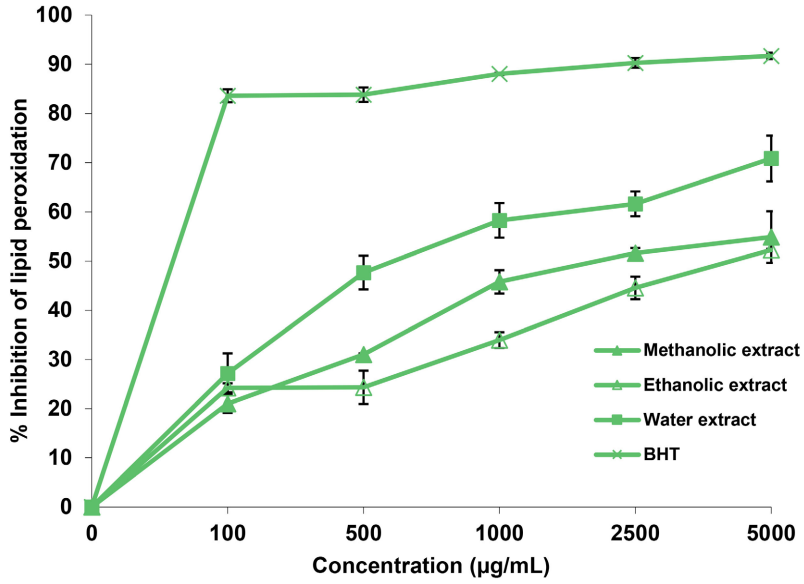

Figure 4. Ability to inhibit lipid peroxidation of *Cistus monspeliensis* extracts. BHT was used as a positive control.

3.7. β-Carotene Linoleic Acid System Activity

In this assay, antioxidant capacity is determined by measuring the inhibition of volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [65]. The antioxidant activity of *C. monspeliensis* extracts is shown in **Figure 5**. All extracts showed lower antioxidant activities than BHT ($p < 0.05$). The antioxidant activity of *C. monspeliensis* extracts followed the order: BHT (96.01%) > EA (24.07%) > EM (20.91%) > EE (19.86%) > EEA (6.03%). Results of this assay showed significant difference ($p < 0.05$) between all *C. monspeliensis* extracts and BHT. This result is not surprising, as certain antioxidant compounds demonstrate limited effectiveness in preventing the bleaching of β-carotene. Similar to the present results, several studies have reported that ascorbic acid, a well-known antioxidant, did not show antioxidant properties in the β-carotene bleaching test [73]-[75]. However, the effectiveness of phenolic antioxidants in inhibiting lipid oxidation in emulsions may vary depending on the polarity, partitioning at oil-water interface, and emulsifier type [76]. In the current body of literature related to the β-carotene/linoleic acid assay, the best inhibition was observed in the non-polar sub-fraction (86.07%) of the methanolic extract of *Eucalyptus sargentii* [77].

A well-known theory in the field concerns the "polar paradox" which illustrates the paradoxical behavior of antioxidants in different media and rationalizes the fact that polar antioxidants are more effective in less polar media while nonpolar antioxidants are more effective in relatively more polar media [78] [79]. Some studies have been conducted to circumvent this problem; one strategy is to significantly reduce the polarity of some molecules by grafting aliphatic chains fat, in order to adjust their position in food or cosmetic matrices [80].

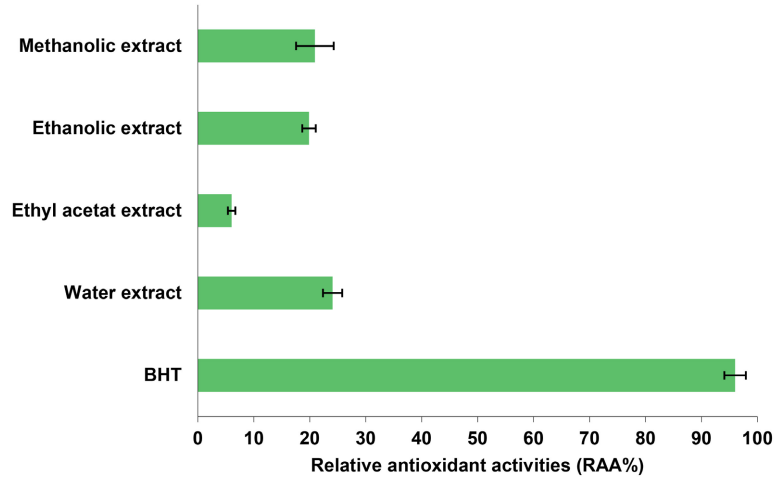

Figure 5. Degradation rates of *Cistus monspeliensis* extracts by β-carotene-linoleate bleaching method. Concentration sample was 2 g/L. BHT was used as a positive control.

We found that the WE and ME of *C. monspeliensis* exhibited significant activity in DPPH and ABTS radical-scavenging activity, reducing power, and ferrous ion-chelating ability at low concentrations. However, these extracts were less effective in terms of TBARS and β-carotene linoleic acid system assays compared to other assays. This justifies the use of various assay approaches for the determination of antioxidant activity, as suggested in the literature [81]. Due to the complex nature of the different phytochemical classes, the antioxidant capacities of plant extracts cannot be evaluated using a single method. Thus, for an extract or fraction to be considered as a good antioxidant, it is not necessary to obtain good results in all tests.

4. Conclusion

This study supports the idea that *C. monspeliensis* may be a good source of natural antioxidants for use by the food industry. WE and ME of *C. monspeliensis* show a good antioxidant activity in the *in vitro* assays applied, which can be attributed to the high phenolic and flavonoid contents. Further identification and isolation of such bioactive components could potentially clarify the antioxidant properties of *C. monspeliensis* and be further exploited for pharmaceutical and food use.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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