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Antioxidant and anti-lipoxygenase activities of extracts from different parts of *Lavatera cretica* L. grown in Algarve (Portugal)

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ABSTRACT

Background: *Lavatera cretica L.* was used in folk medicine as anti-inflammatory among other applications. As inflammation is many times associated with oxidative processes, the aim of the present work was to evaluate the ability of aqueous extracts obtained from different parts of *L. cretica* to prevent oxidation or inflammation using several methods *in vitro*. **Materials and Methods:** The capacity of samples for preventing lipid peroxidation, scavenging free radicals, chelating metal ions, reducing power, and inhibiting lipoxygenase activity was investigated. This last assay also permits to evaluate the anti-inflammatory activity. The quantification of total phenols was performed using Folin-Chiocalteu reagent. **Results:** The highest concentrations of total polyphenols and flavonoids were found in the leaf extract (254.62 ± 6.50 mg gallic acid equivalent/gram; dry weight). Leaf and flower extracts were the most active for scavenging 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt free radicals [Inhibition concentration (IC 50 = 2.88 ± 0.54 and IC50 = 4.37 ± 0.54 µg/mL, respectively)], and leaf extract was also the best for scavenging hydroxyl radicals (IC_{50} = 0.81 ± 0.05 µg/mL). Bract plus sepal extract possessed the best capacity for preventing lipid peroxidation when lecithin liposome was the lipid substrate (IC_{50} = 0.19 ± 0.03 µg/mL) and scavenging superoxide anion radicals (IC_{50} = 1.13 ± 0.48 µg/mL). Leaf and flower extracts were the best lipoxygenase inhibitors (IC_{50} = 0.013 ± 0.0034 µg/mL in both extracts). **Conclusions:** *L. cretica* extracts were able to scavenge free radicals, inhibit lipid peroxidation and lipoxygenase activity. With these attributes, this plant can have an important role in the treatment of neurodegenerative disorders.

Key words: Anti-inflammatory, antioxidant, Cornish mallow, extracts *Lavatera cretica*

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INTRODUCTION

Lavatera cretica L. (syn. *Malva multiflora* (Cav.) Soldano, Banfi and Galasso; syn. *Malope multiflora* Cav.; syn. *Malva pseudolavatera* Webb and Berthel.) (Cornish mallow) belongs to Malvaceae family.[1] *L. cretica* grows in coastal areas of the Near East and Mediterranean region, including North Africa and Macaronesia. It is found on the major Mediterranean islands including at least the Balearic Islands (Malloca, Menorca, Ibiza, Formentera, and Cabrera), Corsica, Sardinia, Sicily, the Maltese archipelago and Crete. It is naturalized in some places of United States of America and South Africa.[2,3]

Since ancient times that *L. cretica* has been used in folk medicine in some regions of Spain as remedy for influenza, digestive, laxative, vulnerary, remedy for relieving of the high respiratory tract, antitussive, and anti-inflammatory.[4-6] Ethnobotanical studies have also revealed that *L. cretica* is used in some provinces of Spain as an edible plant (immature fruits raw as a snack and tender leaves and stems stewed).[6]

In Portugal, *L. cretica* has been also used in folk medicine (anti-inflammatory, analgesic, antiseptic, choleretic, cholagogue, anti-parasite, healing, and laxative).[7] Ethonobotanical studies made in Portugal also demonstrated that in the past, disadvantaged families ate leaves and tender

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stems of *L. cretica* cooked with beans and grains. Children made necklaces with immature fruits. Occasionally, the fruits that formed these same necklaces were consumed by children. Infusion or decoction of *L. cretica* was used by blacksmiths and the pastors to wash the wounds of animals. In humans, infusion or decoctions of this plant was also used in enemas, washes, baths, irrigations, and eye drops. *L. cretica* infusion or decoction was also used as a mouthwash. In the past, the roots of *L. cretica* were given to babies when appeared the first teeth for chewing to soften the pain. In addition, roots favored wear gums required for eruption of teeth. The fresh leaves of *L. cretica* were used to rub the skin after bites of bees or other insects.[8]

Viegi *et al*.[9] made a revision about the use of *L. cretica* extracts to treat gastrointestinal complaints of cattle in Italian veterinary medicine.

Pascual-Villalobos and Robledo[10] found that an acetone extract of *L. cretica* from southeastern Spain was able to cause 100% of mortality of pupae of *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae) after topical application.

Oxidative stress may be defined as an imbalance between cellular production of reactive oxygen species (ROS) and antioxidant defence mechanisms. The processes associated with inflammatory responses are complex and often involve ROS.[11] Therefore, considering that antioxidants and free radical scavengers can exert also an inflammatory effect,[12] the capacity for scavenging free radicals, preventing lipid peroxidation or inhibiting lipoxygenase was the main goal of the present work. This approach was based on the fact the *L. cretica* was described as being used as anti-inflammatory in folk medicine in the Iberian Peninsula[4,5,7] and as far as we know any work regarding the capacity for preventing oxidation *in vitro* was evaluated from different parts of the plant growing in Portugal, more precisely in Algarve.

MATERIALS AND METHODS

Chemicals and reagents

Thiobarbituric acid (TBA); lipoxygenase from soybean; nitroblue tetrazolium (NBT); reduced form of nicotinamide adenine dinucleotide (NADH) and N-phenylmethazonium methosulfate (PMS) were purchased from Fluka, Biochemika, Sigma-Aldrich, Steinheim, Germany. Linoleic acid sodium salt; 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS); 2,2-diphenyl-1-picrylhydrazyl (DPPH); nordihydroguaiaretic acid (NDGA) and 2-deoxyribose were purchased from Sigma-Aldrich, Steinheim, Germany. Trichloroacetic acid was purchased from VWR, Leuven, Belgium. Ferrozine iron reagent hydrate; ethylenediaminetetraacetic acid (EDTA); $FeCl_2.4H_2O$, $FeCl_3$; and potassium persulfate were purchased from Acros organics, New Jersey, USA. L(+)-Ascorbic acid and boric acid were purchased from Merck, Darmstadt, Germany. KH_2PO_4-K_2HPO_4; $FeSO_4.7H_2O$ were purchased from Panreac, Barcelona, Spain. H_2O_2 was purchased from Fisher Scientific, New Jersey, USA. L-α-lecithin of soybean was purchased from CALBIOCHEM, Darmstadt, Germany. Chloroform was purchased from lab-scan, Dublin; Folin Ciocalteu's phenol reagent, was purchased from Panreac Química SA (Barcelona, Spain). Gallic acid; Na_2CO_3; acetic acid; acetic acid; sodium dodecyl sulfate (SDS); KCl; butanol; butylated hydroxytoluene (BHT); mannitol KCl were purchased from Riedel de Haen (Seelze, Germany).

Instrumental

Shimadzu 160UV spectrophotometer (Shimadzu, Kyoto, Japan); rotary evaporator Heidolph 94200 by Bioblock Scientific reduced pressure, Germany. BioTek Synergy™ 4 Hybrid Microplate Reader, Winooski, USA; UV/visible spectrophotometer, Ultrospec 1100 pro, Amersham Biosciences, Uppsala Sweden.

Plant material

Leaves, flowers, bracts plus sepals, and seeds were separated from *L. cretica* after drying in a dark place at room temperature. Plants were collected near the Universidade do Algarve (Portugal) in May 2013 and were identified and authenticated by the staff of the Herbarium of Universidade do Algarve - ALGU (Maria Manuela David and José Manuel Rosa Pinto). A voucher specimen is kept in the ALGU with record number: 14163/ALGU.

Plant extraction

Extracts were prepared as follows: Decoction of 5 g of dried plant material (leaves, flowers bracts plus sepals, and seeds) for 30 min in 50 mL of distilled water. After this operation, the samples were centrifuged for 5 min, at 2000 g at 20°C and the supernatant was removed and kept at −20°C until determination of total phenols and antioxidant activities.

Determination of total phenols (Folin-Ciocalteau)

The extracts total phenol content was determined using the Folin-Ciocalteau reagent and Gallic acid as standard as described by Slinkard and Singleton.[13] The extract sample (0.5 mL) and 2 mL of sodium carbonate (75 g/L) were added to 2.5 mL of 10% (v/v) Folin-Ciocalteau reagent. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm in a spectrophotometer. Tests were carried out in triplicate and the values expressed as gallic acid equivalent.

Antioxidant activity

Thiobarbituric acid reactive species (TBARS). The ability of samples to inhibit malondialdehyde formation, and

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therefore, lipid peroxidation, was determined using a modified TBARS assay. Egg yolk homogenates were used as a lipid-rich medium obtained as described, elsewhere.[14] An aliquot of yolk material was made up to a concentration of 10% (w/v) in KCl (1.15%, w/v). The yolk was then homogenized for 30 s followed by ultrasonication for 5 min. Five hundred microliter of the homogenate and 100 μL of sample were added to a test tube and made up to 1 mL with distilled water; 1.5 mL 20% acetic acid (pH 3.5) and 1.5 mL 0.8% (w/v) TBA in 1.1% (w/v) SDS were then added. This mixture was stirred in a vortex and heated at 95°C for 60 min. After cooling at room temperature, 5 mL of nbutanol were added to each tube, stirred and centrifuged at 3,000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. All of the values were based on the percentage antioxidant index %, whereby the control was completely peroxidized and each oil demonstrated a degree of change; the percentage inhibition was calculated using the formula ($[A_0 - A_1/A_0] \times 100$), where A_0 was the absorbance of the blank sample and A_1 was the absorbance of the sample, was plotted against sample concentrations and IC_{50} was determined (concentration of extract able to prevent 50% lipid oxidation). BHT was used as a positive control.

Inhibition of lipid peroxidation of lecithin liposomes

Liposomes were obtained from 0.4 g lecithin in 80 mL chloroform. This solution was dried and after submitted to nitrogen flux for 30 s. Liposomes were then submitted to vacuum for at least 2 h until complete dryness. Eighty milliliter of phosphate saline buffer 0.01 M, pH 7.0 were added and kept at 4°C until the assay. For the experiment, 250 μL suspension of liposomes was incubated with 25 μL of sample, 400 μL water, 1.5 mL acetic acid 20%, 1.5 mL TBA 0.8 % dissolved in a solution of SDS 1.1%. This mixture was incubated for 1 h at 95°C.[15] After cooling at room temperature, the assay was performed as reported above for TBARS method. BHT was used as a positive control. Tests were carried out in triplicate. The IC_{50} values were determined as reported above.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt$^{\bullet+}$ free radical-scavenging activity. The determination of $ABTS^{\bullet+}$ radical scavenging was carried out as reported by Dorman and Hiltunen.[16] Briefly, the $ABTS^{\bullet+}$ 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 $Abs_{734\,nm}$ to 0.7 at room temperature. Samples (10 μL) were added to 1490 ml $ABTS^{\bullet+}$, the absorbance at 734 nm was read at time 0 (A_0) and after 6 min (A_1). Several concentrations were made and the percentage inhibition $[(A_0\text{-}A_1/A_0) \times 100]$ was plotted against extract content and IC_{50} was determined (concentration of extract able to scavenger 50% of $ABTS^{\bullet+}$ free radical). Tests were carried out in triplicate. BHT and ascorbic acid were used as positive controls.

2,2-diphenyl-1-picrylhydrazyl free radical-scavenging activity. An extract solution (50 μL) of each sample at different concentrations was placed in a cuvette, and 2 mL of 60 μM methanolic solution of DPPH was added.[17] Absorbance measurements were made at 517 nm after 10 min of reaction at room temperature. The values of IC_{50} were determined as reported above. Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

Reducing power determination

The reductive potential of the samples was determined according to the method of Oyaizu[18] with small modifications and described by Bentes *et al.*[19] Each sample was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm against extract concentration in the solution. Tests were carried out in triplicate.

Chelating metal ions

The degree of chelating ferrous ions by samples was evaluated according to the method described by some authors.[20] Samples were incubated with 0.05 mL of $FeCl_2.4H_2O$ (2 mM). The reaction was initiated by adding 5 mM ferrozine (0.2 mL), then after 10 min, the absorbance at 562 nm was measured. An untreated sample served as a control. The percentage of chelating ability was determined according to the following formula: $(A_0\text{-}A_1)/A_0 \times 100$, in which A_0 is the absorbance of the control (without sample) and A_1 the absorbance of the extract. Tests were carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample concentrations. EDTA was used as a positive control.

Hydroxyl radical scavenging activity

The assay of OH-scavenging activity was developed according to Chung *et al.*[21] with small modifications as described by Boulanouar *et al.*[15] 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 TBA (1%) were

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added to the test tube, which was boiled for 10 min. After cooling, its absorbance was measured at 520 nm. The OH-scavenging activity (%) was calculated using the following equation: Inhibition (%) $[(A_o-A_1)/A_o] \times 100$, where A_o 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 extract concentrations. Mannitol was used as a positive control.

Superoxide anion scavenging activity (non-enzymatic method). Measurements of superoxide anion scavenging activity of samples and positive control (BHT) were based on the method previously described.[19] Superoxide anions were generated in a non-enzymatic phenazine methosulfate NADH system by oxidation of NADH and assayed by reduction of NBT. The superoxide anion was generated in 3 mL of phosphate buffer (19 mM, pH 7.4), containing NBT (43 μM) solution, NADH (166 μM) solution and different concentrations of extracts. 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 absorbance at 560 nm was recorded against blank samples. The superoxide anion radical scavenging activity (%) was calculated using the following equation: Inhibition (%) $[(A_o-A_1)/A_o] \times 100$, where A_o 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 extract concentrations. BHT was used as positive control.

5-Lipoxygenase assay

The 5-lipoxygenase assay followed the procedure described by some authors.[22] Briefly, the mixture contained 12.5 μL of each sample dissolved in dimethylsulfoxide, 50 μL of linoleic acid (0.003 g/10 mL) and made up to 1 mL with 0.1 M borate buffer with Tween 0.005%. The reaction was initiated with the addition of 1.5 μL 5-lipoxygenase (0.054 g/mL). The increase in absorbance at 234 nm was recorded for 5 min. The percentage inhibition of enzyme activity was calculated by comparison with the negative control: $\% = [(A_o - A_1)/A_o] \times 100$, where A_o was the absorbance of the blank sample and A_1 was the absorbance of the sample. Tests were carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample concentrations. NDGA was used as a positive control.

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) 18.0 software (SPSS Inc., Chicago, IL). Statistical comparisons were made with one-way analysis of variance followed by Tukey multiple comparisons. The level of significance was set at $P < 0.05$. Correlations between phenol content and antioxidant activity were achieved by Spearman correlation coefficient (r) at a significance level of 99% ($P < 0.01$).

RESULTS AND DISCUSSION

The amount of total polyphenols, measured by Folin-Ciocalteu method, was expressed as gallic equivalent, and the results are expressed in Table 1. The amounts ranged from 43.69 ± 6.50 mg/g dry weight (d.w.), in seed extract to 254.62 ± 6.50 mg/g (d.w.) in leaf extract. Both flowers and bracts plus sepals presented similar concentrations of total phenols (191.38 ± 6.50 and 189.60 ± 6.50 mg/g, respectively). The relative high amounts of phenols found in the extracts obtained from diverse parts of *L. cretica* agree with previous results obtained from other species also belonging to Malvaceae family.[23]

Antioxidant assays in foods and biological systems can be divided into two groups: Those that evaluate lipid peroxidation, and those that measure free radical scavenging ability. The capacity for chelating metal ions is also a method, usually, performed because such metals can catalyze free radical reaction and stimulate lipid peroxidation.[24,25] Therefore, the determination of antioxidant activity of samples must be performed through distinct methods in order to evaluate by which mechanism the sample acts as antioxidant. Although this requisite, they often give inconsistent and conflicting results.[26,27]

Seven assays were used to determine the antioxidant activity of extracts: The capacity for scavenging free radicals (ABTS, DPPH, hydroxyl and superoxide anion free radicals); the capacity for preventing lipid peroxidation using two lipid substrates (egg yolk and liposomes), reducing power and capacity for chelating metal ions.

All samples presented antioxidant activities; nevertheless with different strength [Table 1]. The capacity for preventing lipid peroxidation was significantly better, independent on the plant part, and the lipid substrate used than the positive control BHT. In the TBARS method, there is no significant differences among extracts (IC_{50} values ranging from 0.57 to 2.23 μg/mL in leaf and seed extracts, respectively), in contrast to that observed when liposomes were used as substrate. In this case, the extract obtained from the mix of bracts and sepals (IC_{50} = 0.19 μg/mL) presented the best activity, whereas seed extract had the lowest activity (IC_{50} = 0.98 μg/mL) [Table 1].

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Table 1: Total phenol content, antioxidant, and anti-inflammatory activities of extracts of *Lavatera cretica*

Part of plant	Phenol content*	TBARS**	Liposomes**	DPPH**	ABTS**	Superoxide**	Hydroxyl**	Chelating**	Lipoxygenase**
Leaves	254.62±6.50^{a}	0.57±0.92^{b}	0.32±0.03^{d}	0.70±0.77^{b}	2.88±0.54^{c}	4.95±0.48^{b}	0.81±0.05^{cd}	0.19±0.37^{b}	0.013±0.004^{b}
Flowers	191.38±6.50^{b}	0.37±0.92^{b}	0.41±0.03^{c}	2.13±0.77^{b}	4.37±0.54^{c}	4.01±0.48^{b}	2.14±0.05^{a}	0.49±0.37^{b}	0.013±0.004^{b}
Bract+sepal	189.60±6.50^{b}	0.72±0.92^{b}	0.19±0.03^{e}	0.54±0.77^{b}	6.83±0.54^{b}	1.13±0.48^{c}	0.98±0.05^{c}	0.24±0.37^{b}	0.158±0.004^{a}
Seeds	43.69±6.50^{c}	2.23±0.92^{b}	0.98±0.03^{b}	1.53±0.77^{b}	11.38±0.54^{a}	a)	1.46±0.05^{b}	0.54±0.37^{b}	-
BHT	ND	96.67±0.92^{a}	1.53±0.03^{a}	ND	4.13±0.54^{c}	17.00±0.48^{a}	ND	ND	ND
Ascorbic acid	ND	ND	ND	89.33±0.77^{a}	ND	ND	ND	ND	ND
Mannitol	ND	ND	ND	ND	ND	ND	0.70±0.05^{d}	ND	ND
EDTA	ND	ND	ND	ND	ND	ND	ND	61.00±0.37^{a}	ND
NDGA	ND	ND	ND	ND	ND	ND	ND	ND	0.020±0.004^{b}

*mg GAE/g (d.w.); **IC_{50}=μg/mL. ND: Not determined; -: without activity; a) The activities obtained did not permit to calculate IC_{50} value (see text in results and discussion). TBARS: Thiobarbituric acid reactive species; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 3-ethylbenzothiazoline-6-sulphonic acid; BHT: Butylated hydroxytoluene; EDTA: Ethylenediaminetetraacetic acid; NDGA: Nordihydroguaiaretic acid; IC: Inhibitory concentration

Significant differences in the capacity of extracts for scavenging DPPH free radicals did not exist, only between extracts (IC_{50} = 0.70 μg/mL–2.13 μg/mL, in leaf and seed extracts, respectively) and ascorbic acid (positive control) (IC_{50} = 89.33 μg/mL) [Table 1]. For scavenging ABTS free radicals, leaves and flower extracts had the best capacity without significant differences (2.88 μg/mL and 4.37 μg/mL, respectively). These values were also not significant different to that detected for BHT (4.13 μg/mL). Only seed extracts were poorer as scavenger of ABTS free radicals than the positive control [Table 1]. This extract also had the lowest ability for scavenging superoxide anion radicals. In this case, only 18% of superoxide anion radical was scavenged by the raw seed extract. Such result did not permit to calculate the concentration of seed extract capable to scavenger 50% of that ROS (IC_{50}). The remaining extracts had IC_{50} significantly lower than BHT (17 μg/mL), that is, possessing higher ability for scavenging superoxide anion radicals. Nevertheless, among the remaining extracts, that obtained from bracts and sepals had the best activity (1.13 μg/mL) [Table 1].

Concerning the capacity for scavenging hydroxyl radicals, the extracts reveal to be less adequate than mannitol, the positive control. Only considering the extracts, those obtained from leaves (IC_{50} = 0.81 μg/mL) and bracts plus sepals (IC_{50} = 0.98 μg/mL) had the highest ability for scavenging those free radicals.

All extracts had remarkable higher capacity for chelating metal ions when compared to EDTA (IC_{50} = 61.00 μg/mL), known as chelating agent. All samples had IC_{50} values inferior to 1 μg/mL, without significant differences among them (0.19-0.54 μg/mL) in leaf and seed extracts [Table 1].

Reducing power of extracts was also checked, and differences were observed [Figure 1]. The reducing power of leaf extracts was significantly superior to the remaining extracts, in contrast to the seed extracts that possessed the lowest activity, as can be seen in Figure 1.

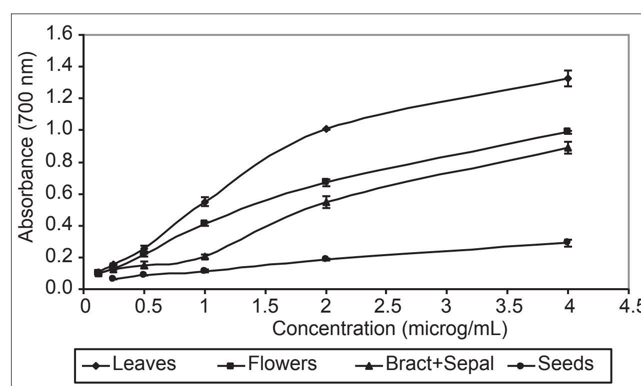

Figure 1: Reducing power of extracts of ***Lavatera cretica***. The bars indicate ± standard error (***n*** = 3)

Lipoxygenase catalyzes the addition of molecular oxygen to fatty acids containing a *cis, cis*-1, 4-pentadiene system. This reaction originates unsaturated fatty acid hydroperoxides. These products are further converted into others that play a key role in inflammatory processes.[28] Hence, compounds which are able to inhibit that enzyme can be considered as antioxidants and possessing anti-inflammatory properties.[29]

The anti-inflammatory activity, measured through the inhibition of lipoxygenase activity, of leaf and flower extracts (IC_{50} = 0.013 μg/mL in both) was similar to that of positive control NDGA (IC_{50} = 0.020 μg/mL) in contrast to the extract obtained from seeds which did not present any activity [Table 1].

In general, the antioxidant activity of plant extracts correlates with phenol content.[15,30] The analysis of the

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Table 2: Spearman correlation coefficients among phenol content and antioxidant and anti-inflammatory activities

Compounds	TBARS	Liposomes	ABTS	DPPH	Superoxide scavenging	Hydroxyl scavenging	Chelating power	Lipoxygenase inhibition
Phenols	−0.601*	-	−0.832**	-	-	-	−0.804**	-

*Correlation is significant at the $P<0.05$ level; **Correlation is significant at the $P<0.01$ level. -: Not significant; TBARS: Thiobarbituric acid reactive species; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 3-ethylbenzothiazoline-6-sulfonic acid

correlation between the total phenolic compounds and antioxidant and anti-inflammatory activities showed significant dependence in the case of ABTS scavenging ability ($r = -0.832$; $P < 0.01$), lipid peroxidation prevention when egg yolk was as lipid substrate ($r = -0.601$; $P < 0.05$) and chelating activity ($r = -0.804$; $P < 0.01$) [Table 2]. The activities found in the other methods which did not correlate with phenol content may be due to other nonphenolic components also present in extracts. In addition, the assay for quantifying phenols is nonspecific not only to polyphenols but to any other substance that could be oxidized by the Folin reagent.[23,31] Hence, these factors may be responsible for the low or even absence of correlation between some activities and phenol content. In other species belonging to Malvaceae family,[25] the authors also found a low correlation between total phenol content and antioxidant activity.

CONCLUSION

The aqueous extracts of *L. cretica* had a remarkable capacity for preventing lipid peroxidation, scavenging free radicals, and inhibiting lipoxygenase activity. In some cases, these abilities were even substantially superior to the positive controls. Nevertheless, and in some cases (liposomes, ABTS, superoxide, hydroxyl, and lipoxygenase), those activities were dependent on the part of the plant used. In Portugal, this plant has been considered of low interest, and the results found in the present work reveal that this species has a great potential as a medicinal plant.

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