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Bioactive compounds, anti-oxidant and anti-inflammatory effects of carob fruit and leaves (*Ceratonia siliqua*)

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ABSTRACT. The present study aimed to make a phytochemical study and to evaluate the *in vitro* antioxidant and anti-inflammatory activities of the crude extracts of two parts (leaves and fruit) of *Ceratonia siliqua*. The polyphenols extraction step was followed by the determination of polyphenols and flavonoids contents. The antioxidant activity was evaluated using the DPPH test and the ferric reducing power. Protein denaturation (BSA) and red blood cell membrane stabilization assays were used for the anti-inflammatory activity. Phytochemical assays showed that leaves extract was richer in polyphenols and flavonoids compared to fruit. The DPPH antiradical activity and the ferric reducing power revealed that the methanol extract of the leaves has the highest antioxidant activity compared to the fruit. For the anti-inflammatory activity, the results also showed that the crude extract of the leaves has a powerful activity against denaturation of proteins (BSA), and has an important protective effect against membrane hemolysis of red blood cells compared to fruit extract. The obtained results suggested that *Ceratonia siliqua* is an effective antioxidant and anti-inflammatory agent.

Keywords: *Ceratonia siliqua*; stress; inflammation; polyphenols.

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Introduction

The overproduction of reactive oxygen species (ROS) beyond the antioxidant capacities of biological systems results in oxidative stress that is involved in the occurrence of several diseases including inflammation (Battu et al., 2011). This is a defensive response of the immune system, whereby the body mounts an immune response to an infectious agent or antigen, as well as cellular damage. It is a fundamental biological process and the most common sign of disease. Thus, inflammation and oxidative stress are pathophysiological events closely related to a number of chronic diseases, including diabetes, hypertension and cardiovascular disease, neurodegenerative diseases, cancer and aging (Biswas, 2016).

Anti-inflammatory therapy is generally conducted by synthetic molecules of non-steroidal or steroidal anti-inflammatory types (corticosteroids), whose side effects are sometimes serious and pose a major problem in their clinical use. Therefore, to overcome their toxicity, the development of new anti-inflammatory medicines is still needed and the natural product such as medicinal plants could potentially serve as a precursor in the production of new drugs to treat inflammation with reduced or no side effects (Lachkar et al., 2016).

Medicinal plants usually contain mixtures of different chemical compounds (bioactive secondary metabolites such as polyphenols and flavonoids) that can act individually, additively or synergistically to improve health (Gurib-Fakim, 2006). *Ceratonia siliqua* is a tree belonging to the Fabaceae family; it is typical of the Mediterranean region. It is characterized by its ecological and economic importance and also by its medicinal and therapeutic properties (Ghanemi & Belarbi, 2021) including anti-inflammatory, antimicrobial, anti-diarrheal, antioxidant, anti-ulcer, anti-constipation etc (Rtibi et al., 2017).

To the best of our knowledge, there are few reports carried out on the evaluation of the antioxidant and anti-inflammatory activities of polyphenols from *Ceratonia siliqua*. Therefore, the aim of the present study is

to determine the polyphenolic content of methanol extracts from two parts of the plant (leaves and fruit) and to evaluate their *in vitro* antioxidant and anti-inflammatory potentials.

Material and methods

Plant material collection

Ceratonia siliqua leaves were collected in March 2022 from Ouled Yahia region, Jijel (Algeria). After being thoroughly cleaned, fresh, intact leaves of the plant were dried in the open air then in an oven, at a temperature of 40°C, until the stabilization of weight. Air-dried leaves were ground using an electric grinder into a fine powder and then kept in tinted glass bottles to ovoid the oxidization of their compounds.

Polyphenols extraction

The extraction of polyphenols was carried out at ambient temperature for 72 hours by maceration in methanol-water 80/20 (v/v) at a solid-liquid ratio 1/10 (w/v) with continuous stirring. The solvent was then filtered, de-fatted by hexane referring to the procedure of Yu et al. (2005). The process was repeated thrice, and the recovered methanolic phase was concentrated using a rotary evaporator at 40°C under reduced pressure. The extraction yield (the ratio between the weight of the dry extract in grams and the weight of the powder plant used in grams), expressed as a percentage, was calculated according to Stanojevic et al. (2009). The dried extract was stored in the dark at 4°C until used.

Total phenolic content

Determination of total polyphenols in both plant extracts was carried out using the Folin Ciocalteu method (Heilerova et al., 2003). It is a colorimetric method that allows the analysis of organic compounds that have hydroxylated aromatic cycles. Briefly, 0.2 mL of the two dilute raw extracts (250 µg mL^{-1}) were added with 1.5 mL of the Folin Ciocalteu reagent (1/10). The mixture is left to stand for 5 minutes in the dark. Subsequently, 1.5 mL of Na_2CO_3 solution (7.5%) was added to the set. After 60 minutes of incubation at room temperature and dark, the absorbance was measured at 750 nm against a blank using a spectrophotometer (Spectro® 50 plus). A calibration curve is performed in parallel under the same operating conditions using gallic acid. Total phenol contents were expressed as mg Gallic Acid Equivalents (GAE) per g of crude extract (CE).

Total flavonoids content

Estimates of flavonoid content are commonly based on the use of aluminum chloride ($AlCl_3$) (Huang et al., 2004). To perform the assay, 1.5 mL of each extract (2 mg mL^{-1}) was added to 1.5 mL of $AlCl_3$ solution (2%). The different solutions were incubated at room temperature in the dark for 30 minutes and the absorbance was read at 430 nm. A calibration curve performed by quercetin at different concentrations under the same operating conditions as the samples was used for flavonoid quantification. The flavonoid content was expressed in milligrams quercetin equivalent per gram of raw extract (mg EQg^{-1} EB).

Assessment of *in vitro* anti-inflammatory activity

Inhibition of protein denaturation method

The anti-inflammatory activity of *Ceratonia siliqua* was studied by using the inhibition of albumin denaturation technique, which was studied according to Mizushima and Kobayashi (1968) followed with minor modifications. 500 µL of 1% BSA was added to 100 µL of plant extract (25 - 200 µg mL^{-1}). A solution of HCl (1N) was used to adjust the pH of the reaction mixture. This mixture was then incubated at 37ºC for 20 minutes and then heated to 51ºC for 20 minutes. The turbidity was measured at 660 nm after the samples were cooled to room temperature. Diclofenac sodium was taken as a standard and the experiment was performed in six replicates. Protein denaturation inhibition was determined as follows:

$$\% \text{ inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample}) \text{ X } 100}{\text{Abs control}}$$

where: Abs Control is the absorbance without sample; and Abs Sample is the absorbance of sample/standard.

The concentration of the extract/drug causing 50% inhibition (IC_{50}) of BSA was calculated from the linear regression curves.

HRBC membrane stabilization method

Human red blood cell (HRBC) membrane stabilization assay was determined using the method developed by Shinde et al. (1999).

The blood was collected from a healthy human volunteer who had not taken any NSAIDs (Non Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to heparinized centrifuge tubes. The collected blood was mixed with an equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and, 0.42% sodium chloride in distilled water). The blood was subjected to centrifugation at 3,000 rpm for a period of 10 minutes, after which the packed cells were rinced three times with isosaline (0.85%, pH 7.2). The blood volume was measured and reconstituted as 10% v/v suspension with isosaline. Different concentrations of extract (25 - 200 µg mL^{-1}), Diclofenac sodium as standard, and control (distilled water instead of hyposaline to produce 100% hemolysis) were separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of 10% HRBC suspension. All the centrifuge tubes containing the reaction mixture were incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 min. The supernatants absorbance was recorded at 560 nm. The experiment was performed in six replicates and the percentage inhibition of haemolysis or membrane stabilization percentage was calculated as follows:

$$\% \text{ inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample}) \text{X } 100}{\text{Abs control}}$$

The concentration of the extract/drug causing 50% inhibition (IC_{50}) of erythrocyte haemolysis was calculated from the linear regression curves.

Assessment of *in vitro* antioxidant activity

DPPH free radical scavenging assay

The radical scavenging activity of the extract was determined using the DPPH assay as described by Brand-Williams et al. (1995). Aliquots (100 µL) of various concentrations of the extract (25 - 200 µg mL^{-1}) in methanol were added to 2.9 mL of a 0.025 g L^{-1} methanol solution of DPPH. The mixtures were shaken vigorously and left standing at room temperature for 30 minutes in the dark. The decrease in the purple coloration of the reaction mixtures due to the bleaching of the DPPH color was read using a UV-spectrophotometer at 517 nm. The DPPH· radical scavenging ability was calculated using the formula:

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A_1}{A_0}. 100$$

where:

A_0: the absorbance of the control (containing all reagents except the sample) at 30 min.

A_1: the absorbance of the sample at 30 min.

All tests were run in six replicates and averaged. Ascorbic acid was used as positive control.

Antiradical activity was also calculated as the EC_{50}, defined as the concentration of the extract generating 50% of the maximum dose response calculated with linear regression.

Ferric reducing power assay

The reducing power of the extract and the standard (ascorbic acid) were determined according to the method described by Oyaizu (1986).One milliliter of the extract (50, 100, 250, and 500 µg mL^{-1}) was mixed with phosphate buffer (0.2 M, pH 6.6) and one milliliter potassium ferricyanide [$K_3Fe(CN)_6$] (1%).The reaction mixture was incubated at 50°C for 20 minutes and then was acidified with 1 mL of trichloroacetic acid (TCA) (10%).The resulting solution was centrifuged for 10 minutes at 3,000 rpm. Finally, the upper layer of solution (1.5 mL) was mixed with distilled water (1.5 mL) and ferric chloride ($FeCl_3$) solution (150 µL, 0.1%), then the absorbance was measured at 700 nm and compared to the standard (ascorbic acid). An increase in absorbance indicated increased reducing power. The assay was carried out in six replicates.

Statistical analysis

Each experiment was run six times (n=6), and the results are shown as mean ± standard deviation (SD). ANOVA was used to analyze the data, and then the Tukey-Kramer HSD test was performed (JMP version 7.0 Software). Statistics were considered significant if $p < 0.05$.

Results and discussion

Extraction yield

The extraction yield varies from one part of the plant to another. The fruit extract gave the best yield: 40.04% against 17.74% for the leaves.

According to Spizzirri et al. (2022), the yields of leaf extracts from two varieties of *Ceratonia siliqua* (Amele and selvatica) harvested in the southern region of Italy were 14.7 and 7% respectively using ultrasonic extraction in ethanol. Based on the results of Rtibi et al. (2016), the yield of aqueous leaf extract collected in the Tabarka region (northwest Tunisia) was 10%. These results are lower than those obtained in our study. In the Ydjedd et al. (2017) study, the extraction yield of carob fruit grown in Bejaia, Algeria, was 0.80% for ethyl acetate extraction versus 53.06% for acetone extraction. Another study by Alabdallat and Bilto (2013) on the fruit of *Ceratonia siliqua* collected in Jordan showed a yield of 23.2% using methanol as extraction solvent. This variability of yield depends strictly on the polarity of the solvent, extraction techniques, temperature, pressure, time and the chemical composition of the sample.

Total phenolics and flavonoids contents

Total phenolic contents of the two *Ceratonia siliqua* extracts were analyzed and presented in Table 1. Our results show that the leaf extract of *Ceratonia siliqua*, has the highest polyphenols content (141.68 ± 7.39 mg $EAGg^{-1}$ EB) compared to that of the fruit which had a content of 45.59 ± 2.20 mg $EAGg^{-1}$EB.

Table1. Total phenols content in *Ceratonia siliqua* extracts.

Total phenols content	(mg $GAEg^{-1}$ CE)
Leaves extract	141.68 ± 7.39
Fruit extract	45.59 ± 2.20

Values are means± SD, n = 6.

The study conducted by Abidar et al. (2020) on the aqueous extract of *Ceratonia siliqua* leaves showed that the total polyphenols content was 52.95 ± 0.141 mg $EAGg^{-1}$ dry weight. Thus, in the study of Hsouna et al. (2011), the polyphenols content in the raw leaf extract/water fraction was 130 ± 5.62 mg $EAGg^{-1}$ PS. Another study by Custódio et al. (2015) showed that the polyphenols content of leaf decoction was 65 mg $EAGg^{-1}$ PS. The results obtained in the work of Spizzirri et al. (2022) carried out on two varieties of carob tree by different extraction techniques were varied between 29.22 ± 0.17 and 313.04 ± 0.36 mg $EAGg^{-1}$ PS. For the fruit of *Ceratonia siliqua*, a study by Roseiro et al. (2013) showed that the polyphenols content varies between 5.8 ± 0.3 and 20.4 ± 1.8 mg $EAGg^{-1}$ PS depending on the extraction method and solvent. Based on the study by Makris and Kefalas (2004), the polyphenols content of methanol extract was 3.38 ± 0.52 mg $EAGg^{-1}$ PS. Custódio et al. (2015) showed polyphenols at 8.2 ± 0.1 mg $EAGg^{-1}$ PS using decoction.

Similarly, total flavonoids contents equivalent to quercetin (QE) were calculated and presented in Table 2. According to the results, we noted that the leaves extract of *Ceratonia siliqua* is also the richest in flavonoids with a content of 48.12 ± 1.87 mg EQg^{-1} EB compared to the fruit (12.46 ± 2.23 mg EQg^{-1} EB).

Table2. Total flavonoids contents in *Ceratonia siliqua* extracts.

Total flavonoids content	(mg $GAEg^{-1}$ CE)
Leaves extract	48.12 ± 1.87
Fruit extract	12.46 ± 2.23

Values are means± SD, n = 6.

Based on the study of Dallali et al. (2018) on *Ceratonia siliqua* leaves collected from three different sites in the northeast region of Tunisia, the flavonoids content was from 3.42 ± 0.55 to 7.42 ± 0.22 mg EQg^{-1} PS. According to Abidar et al. (2020), the flavonoids content of aqueous leaf extract was 25.35 ± 0.124 mg EQg^{-1} PS. In addition, a study by Hsouna et al. (2011) showed values between 21.71 ± 8.71 and 193.3 ± 3.07 mg EQg^{-1} PS using four different extraction solvents.

For the fruit of *Ceratonia siliqua*, a study by Ydjedd et al. (2017) showed a flavonoids content of 1.52 ± 0.14 mg EQg^{-1} PS using ethyl acetate as extraction solvent. In addition, another study by Bentahar et al. (2012) showed a flavonoids content of 0.033 ± 0.01 mg EQg^{-1} PS whose extraction solvent was distilled water. Based on the results of Hsouna et al. (2012) the flavonoid content of methanol extract was 24.6 ± 2.4 mg EQg^{-1} PS.

The difference in phenolic contents including flavonoids can be attributed to several factors namely the extraction and the quantification methods. In addition, climatic and environmental factors (geographical area, drought, temperature, altitude, sunshine and precipitation), variety, origin, the period of harvest and the stage of development as well as the various diseases that may affect the plant, the maturity of the plant can also influence the polyphenols and flavonoids contents (Locatelli et al., 2010; Bentahar et al., 2012; Dallali et al., 2018). Genetic factors and plant growth conditions can play an important role in the formation of secondary metabolites (Islam et al., 2003).

Anti-inflammation assays

Inhibition of albumin denaturation

The anti-inflammatory activity of extracts was investigated by inhibition of heat-induced albumin denaturation. As presented in Table 3, the extracts and standard have the ability to inhibit heat-induced BSA denaturation in a dose dependent manner. Maximum inhibition of extracts was observed at a concentration of 200 µg mL^{-1} (63.22±0.71% and 47.89±0.68 for leaves and fruit extracts respectively) and the IC_{50}value were found to be 130.07±1.57 and 198.39±2.4 µg mL^{-1} for leaves and fruit extracts, respectively. Diclofenac sodium showed comparatively higher anti-inflammatory activity (73.02±0.48%) with a lower IC50 value (71.75±2.11 µg mL^{-1}) at the same concentration.

Table 3. Effect of *Ceratonia siliqua* extracts and standard on protein denaturation and HRBC membrane stabilization.

Concentrations	Protein denaturation(% inhibition)			Membrane stabilization (% protection)		
(µg mL^{-1})	Leaves extract	Fruit extract	Standard	Leaves extract	Fruit extract	Standard
25	23.12±0.71^{f}	13.12±0.71^{g}	37.49±0.41^{e}	26.55±0.77^{f}	17.12±0.71^{g}	35.55±0.43^{e}
50	37.28±0.70^{e}	25.28±0.7^{f}	48.00±0.48^{d}	38.89±0.50^{d}	29.28±0.70^{f}	54.09±0.15^{c}
100	46.05±0.73^{d}	37.11±0.64^{e}	58.57±0.76^{c}	53.08±0.70^{c}	41.11±0.64^{d}	63.49±1.54^{b}
200	63.22±0.71^{b}	47.89±0.68^{d}	73.02±0.48^{a}	69.24±0.56^{a}	51.89±0.68^{c}	71.76±1.55^{a}
IC_{50} (µg mL^{-1})	130,07±1,57	198,39±2,4	71,75±2,11	107.28±1.24	176.65±1.99	59.06±0.84

The results are expressed as mean ± SD (n = 6). According to Tukey HSD Test, values followed by different subscripts are significantly different ($p < 0.05$). Linear regression analysis was used to calculate IC_{50} value.

Protein denaturation is a process in which proteins lose their tertiary and secondary structure by applying stress or an external compound, such as a strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most proteins lose their biological function when denatured (Sivaraj et al., 2017). Protein denaturation is a well-documented cause of inflammation (Leelaprakash & Dass, 2011).

As part of the study of the mechanism of anti-inflammatory activity, the ability of *C. siliqua* extracts to inhibit protein denaturation was evaluated. The results show that these extracts were effective in inhibiting heat-induced albumin denaturation.

Based on our results, we find that both crude extracts (leaves and fruit) of *Ceratonia siliqua* were able to control the denaturation of proteins and thus inhibit the production of auto-antigens. The denaturation mechanism may involve alteration of electrostatic, hydrogen, hydrophobic and disulfide bonds (Kar et al., 2012). The inhibitory activity of BSA denaturation can be attributed to the presence of different bioactive compounds such as flavonoids in extracts. It can be concluded that the extracts have an anti-inflammatory effect.

Membrane stabilization test

Data in Table 3 are the percentage of HRBC membrane protection against hyposaline induction by methanol extracts and Diclofenac sodium. Both plant extracts and standard showed inhibition of heat-induced hemolysis. This data showed the higher the concentrations of the extract, the greater the HRBC membrane protection. Both extracts tested provided human red blood cells with protection against hypotonic hemolysis that was lower than the reference molecule (diclofenac). The percentage of membrane protection by the leaf extract (69.24±0.56%) is higher compared to the fruit extract (51.89±0.68%) and close to that of the reference anti-inflammatory (71.76±1.55%).

Red blood cell membrane stabilization was used as a method to study anti-inflammatory activity *in vitro* because the erythrocyte membrane is analogous to the lysosomal membrane (Anosike et al., 2012) and its stabilization implies that the extracts may well stabilize the lysosomal membranes. Lysosome stabilization is important to limit the inflammatory response by preventing the release of activated neutrophil lysosomal constituents, such as bacterial enzymes and proteases, this causes additional tissue inflammation and damage

during extracellular release (Leelaprakash & Dass, 2011). Lysosomal enzymes released during inflammation produce various disorders. The extra-cellular activity of these enzymes is thought to be related to acute or chronic inflammation. Non-steroidal drugs either inhibit these lysosomal enzymes or stabilize the lysosomal membrane (Vadivu & Lakshmi, 2008).

Also erythrocytes are considered major targets for free radical attack due to the presence of both a high concentration of polyunsaturated fatty acids on the membrane and the oxygen transport associated with redox active hemoglobin molecules, which are powerful promoters of reactive oxygen species (Carvalho et al., 2010). According to Namoune et al. (2018) polyphenols are capable of protecting erythrocytes from active stress or increasing their resistance to oxidant damage.

According to our results, the extract of the leaves shows a stabilization of the erythrocyte membrane more important than the fruit extract. This activity may be due to the strong presence of polyphenols and flavonoids which have anti-inflammatory properties.

Antioxidant assays

DPPH radical scavenging assay

The results on the DPPH free radical scavenging activity of the extract and standard are shown in Table 4. The preparations were able to reduce the stable free radical DPPH in a dose- dependent manner. The extracts showed maximum activities of 64.43±0.53% and 44.66±0.55 for leaves and fruit extracts, respectively at the concentration of 200 µg mL^{-1}, whereas the positive standard exhibited 78.87±0.76% inhibition at the same concentration value. The results showed that *Ceratonia siliqua* extracts have a good DPPH radical scavenging effect with an IC50 value of 121.48±1.08 and 216.91±3.33 µg mL^{-1} for leaves and fruit extracts, respectively, that are higher than that of positive control ascorbic acid (75.96±1.8 µg mL^{-1}).

Table 4. DPPH free radical scavenging activity of *Ceratonia siliqua* extracts and standard.

Concentrations (µg mL^{-1})	DPPH(% inhibition)		
	Leaves extract	Fruit extract	Ascorbic acid
25	$28.71±0.68^{g}$	$12.81±0.61^{i}$	$33.58±^{f}$
50	$36.04±0.53^{f}$	$22.49±0.85^{h}$	$40.48±0.44^{e}$
100	$48.78±0.30^{c}$	$35.23±0.68^{f}$	$65.34±0.78^{b}$
200	$64.43±0.53^{b}$	$44.66±0.55^{d}$	$78.87±0.76^{a}$
IC_{50} (µg mL^{-1})	121.48±1.08	216.91±3.33	75.96±1.8

Data are presented as mean ± SD (n = 6). According to Tukey HSD Test, values followed by different subscripts are significantly different ($p < 0.05$). Linear regression analysis was used to calculate IC_{50} value.

Hsounaet al. (2012) showed that *Ceratonia siliqua* leaves have a scavenger effect of DPPH free radicals (IC_{50}) of 41.01, 1.80, and 8.65 µg mL^{-1} using dicloromethane, ethyl acetate and water as extraction solvents respectively. Also, the study of Custódio et al. (2009) on six Portuguese female varieties of *Ceratonia siliqua* showed that the IC_{50} of the leaf extract varied between 94.6 and 273.2 µg mL^{-1}. According to Ydjedd et al. (2017) the IC_{50} of the fruit extract was 992.04 and 795.32 µg mL^{-1} whose extraction solvents were ethyl acetate and acetone, respectively. Another study showed radical activity in the range of 165.08 and 1618.70 µg mL^{-1} for ethyl acetate and diethyl ether extracts (Gregoriou et al., 2021).

These results show that antiradicalar activity against DPPH is influenced by the choice of extraction solvent and it is correlated with the levels of polyphenols and flavonoids in plant extracts (Mariod et al., 2009; Locatelli et al., 2010). Polyphenols appear to be effective donors of hydrogen to the DPPH radical due to their ideal chemical structure (Turkmen et al., 2007). The strong antioxidant activity of the leaves of *Ceratonia siliqua* is therefore related to their high content of total phenols and flavonoids compared to the fruit. The trapping capacity of DPPH in plant extracts increases as the level of OH- groups in aromatic cycles increases (Shahwar & Raza, 2012). This conclusion is supported by published reports which indicate that phenolic substances generally well correlate with scavenging activity on DPPH radicals (Boutennoun et al., 2017; Boutennoun et al., 2019).

Ferric reducing power activity

The reductive capabilities of *Ceratonia siliqua* extracts compared to the standard ascorbic acid are presented in Table 5. Similar to the antiradical activity, the reducing power of the extract increased with increasing concentrations. For leaves extract, the ferric reducing power is statistically comparable to that of ascorbic acid.

Table 5. Ferric-reducing power of *Ceratonia siliqua* extracts and ascorbic acid.

Concentrations ($\mu g\ mL^{-1}$)	Absorbance at 700 nm		
	Leaves extract	Fruit extract	Ascorbic acid
25	0.198 ± 0.01^{f}	0.153 ± 0.02^{g}	0.211 ± 0.05^{f}
50	0.322 ± 0.02^{d}	0.252 ± 0.02^{e}	0.334 ± 0.01^{d}
100	0.493 ± 0.01^{c}	0.391 ± 0.01^{c}	0.491 ± 0.01^{c}
200	0.615 ± 0.02^{a}	0.501 ± 0.01^{b}	0.612 ± 0.02^{a}

Data are presented as mean ± SD (n = 6). According to Tukey HSD Test, values followed by different subscripts are significantly different ($p < 0.05$).

Chelation of transition metals is one of the main strategies used to study the antioxidant activity of plant extracts (Mathew & Abraham, 2006). Transition metal ions play a critical role in the oxidation process via the Fenton reaction (Hinneburg et al., 2006).

Our results show that there is a good correlation between the reductive power of *Ceratonia siliqua* extracts and their total polyphenols and flavonoid contents. The leaves have the highest reductive power compared to the fruit, probably due to their high content of total polyphenols and flavonoids. This is consistent with several reports that have shown a close relationship between total phenolic content and the reducing power of plants (Boutennoun et al., 2019). The antioxidant power of phenolic compounds is due to their high redox potential, which allows interaction as hydrogen donors, reducers and singlet oxygen extinguishers ($^1O_2$) (Loizzo et al., 2009). Furthermore, the structural composition of phenolic compounds is also implicated in their antioxidant activity. The presence of hydroxyl groups in the aromatic cycles of the flavonoids in the 3', 4' and 5' position increases their antioxidant power. In addition, phenolic compounds with two adjacent hydroxyl groups may participate in the chelation process of transition metal ions such as iron and copper (Miguel et al., 2010).

Conclusion

The outcomes from the present study exhibited that the methanolic extracts of *Ceratonia siliqua* leaves showed significant *in vitro* antioxidant and anti-inflammatory properties. Our results confirm the potential role of the studied plant as a remedy for the treatment of inflammation and related diseases. This plant can be a low-cost source of essential bioactive compounds with potential for herbal medicine development. Further studies are necessary to purify the bioactive compounds that are responsible of the biological activities.

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