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Bioactive metabolites involved in the antioxidant, anticancer and anticalpain activities of *Ficus carica* L., *Ceratonia siliqua* L. and *Quercus ilex* L. extracts

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ABSTRACT

Ficus carica L., *Ceratonia siliqua* L. and *Quercus ilex* L. are natural products that are used as an advantageous rich source of bioactive compounds of high economic value because of its use in cosmetic, pharmaceutical and agriculture industries. These crops were studied for its phytochemical contents and were investigated for antioxidant activities and its effects on reactive oxygen species (ROS) production, calpain activity and antiproliferative effects. Results showed that extremely high total contents of phenolics, flavonoids and *ortho*-diphenols were detected in *Quercus ilex*, while proanthocyanidin level was higher in *Ficus carica*. *Ceratonia siliqua* pods showed more carotenoids and had the highest lightness value (L*). *Quercus ilex* and *Ceratonia siliqua* extracts were very effective in scavenging free radicals. The best hydroxyl radical scavenging activity was attributed to *Quercus ilex* with 80.51 ± 0.20% while nitric oxide assay showed no significant differences between extracts. *Ficus carica* presented a higher reducing ability with 638.23 ± 0.43 mg gallic acid equivalents/100 g, while *Quercus ilex* was very potent in reducing power assays. Percentages of metal chelating capacities of *Quercus ilex* and *Ficus carica* extracts were 87.87 ± 0.34 and 73.17 ± 0.16%, respectively. These two samples were also able to scavenge hydrogen peroxide efficiently. At 250 μg/mL, *Quercus ilex* presented the best xanthine oxidase inhibition, 89.81 ± 0.36%. *Ceratonia siliqua* and *Quercus ilex* extracts presented the best capacities of ROS inhibition and reduced cell viability in a concentration dependent manner. All samples decreased calpain activity. These extracts could be further exploited as a source of natural products with antioxidant and anticancer effects.

1. Introduction

Crop products represent a major focus for drug development and industry and it holds a significant share in drug-market all over the globe. *Ficus carica* is a tree that is classified in the Moraceae family, it represents one of the most important crop cultivated in the worldwide. Its figs (*Ficus carica*) harvested in July and August are vey consumed due to its high contents in sugar, source of energy, and antioxidants components like phenolic compounds and vitamins. Figs are also known for its various therapeutic properties such its use in traditional medicine for the treatment of cardiovascular, respiratory and anti-inflammatory disorders (Çalişkan and Aytekin Polat, 2011). Another tree native to the Mediterranean is *Ceratonia siliqua*. This evergreen plant is belonging to the Fabaceae family. It has been cultivated for a long time and the most used part is pods (*Ceratonia siliqua*). These are very exploited by industry to produce locust bean and carob bean gum (Ayaz et al., 2007). The genus *Quercus* has been the subject of intense research due to its important role in industry section. More than 300 species were found in the Mediterranean area. *Quercus ilex* roots barks are used in the Alge-

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rian folk medicine to treat gastropathies. Acorns (*Quercus ilex*) were mainly used for animal consumption and were found to be rich in tanins (Cantos et al., 2003; Karioti et al., 2010; Custódio et al., 2013).

To improve and maintain the products, manufacturers are actually looking for effective natural antioxidants with negligible side effects. Phenolic compounds are among the compounds that respond more to the satisfaction. The objectives are: (i) customer satisfaction and protect their health by consuming products containing natural antioxidants, (ii) to find another source of natural antioxidants for pharmaceutical and cosmetic industries, (iii) to find ingredients to improve the sensory and nutritional quality of products and its possible use for the formulation of new products (Feng et al., 2015).

The importance of antioxidants lies in its ability to inhibit the production of reactive oxygen species. These compounds are able to exercise a scavenger effect on free radical species derived from oxygen or nitrogen and neutralize non-radical species such as hydrogen peroxide. In some physiopathologic circumstances, there is an excessive production of free radicals leading to the occurrence of oxidative stress. This later is related to the appearance of several diseases such as cardiovascular disease, Alzheimer's and cancer (Park et al., 2011). Several studies were performed on antioxidants in the sense that the intake of these compounds leads to the improvement of health and reduces the rate of mortality associated with diseases caused by oxidative stress (Marrelli et al., 2012).

Despite the large body of literature on the antioxidant and phenolic contents of figs, carob pods and acorns, several aspects of its biological activities remain unexplored. In that framework, the aims of this work were focused on the determination of the antioxidants composition of *Ficus carica* figs, *Ceratonia siliqua* pods and *Quercus ilex* acorns, to quantify and identify the main carotenoids present of these, and to investigate its antioxidant activities. There are several antioxidants which differ according to the types, structures, mode of action and the reactivity and that is why several tests on the antioxidant activity were performed. Other complementary assays are also used to estimate the total antioxidant capacity of the studied extracts. Having in mind the potential of *Ficus carica*, *Ceratonia siliqua* and *Quercus ilex* as source of bioactive compounds and the lack of bioactivity reports on *Quercus ilex*, this work also evaluated for the first time the anti-inflammatory and anticalpain activities of its extracts and its cytotoxic effects on human glioblastoma cancer cells.

2. Material and methods

2.1. Chemicals

Methylthiazolyldiphenyl-tetrazolium bromide (MTT) and lucigenin were provided from Sigma-Aldrich (France) and the Eagle's Minimal Essential Medium (EMEM) was purchased from Invitrogen. L-glutamine, sodium pyruvate, Fetal Bovine Serum (FBS) and trypsin- ethylene diamine tetra-acetic acid (EDTA) were purchased from Invitrogen (Scotland-UK). Antibiotics streptomycin and penicillin were obtained from GIBCO (Cergy- Pontoise, France). The chromatographic solvents were methanol, acetonitrile, ethyl acetate (HPLC grade, procured from Merck, Darmstadt,Germany). Water was purified in a NANOpure®DIamond™system (Barnsted Inc., Dubuque, IO). Standards β-carotene, pheophytins a and b, luetin, β-cryptoxanthin, Chlorophylls a and b, antheraxanthin and zeinoxanthin were obtained from Sigma-Aldrich (Germany).

2.2. Samples preparation

Samples were harvested from three locations in Bejaia city (Algeria): carob pods (*Ceratonia siliqua*) from Ighil-Yesli, holm oak acorns (*Quercus ilex*) from Aichoune and white figs (*Ficus carica*) from Beni Maouche. The collection was done in clean places, away from pollution impact. Each specimen within the harvest site had been a random sampling on several samples. Botanical identification was made by the member of laboratory of Botany (University A. Mira of Bejaia). Voucher specimens were preserved at the Herbarium of Natural History Museum of Aix-en-Provence, France (*Ceratonia siliqua*: D-PH-2013-37-8; *Ficus carica*: D-PH-2013-37-9 and *Quercus ilex*: D-PH-2013-37-10). Healthy and uninfected samples were selected. Seeds were removed from ripe carob pods and pulp was dried in a ventilated oven (40 °C) until a constant weight of samples. Mature acorns were husked manually to separate the kernels and pericarp and the pulp was crushed and milled to a powder. The resulting millings from the studied crops were sifted in order to select the powders having a diameter smaller than 500 μm. Dried figs were cut and triturated into small pieces. Powders from the studied crops were retained and conserved in smoked glass, sealed and stored away from light and moisture for subsequent uses.

2.3. Extraction procedure

The procedure of extraction was to mix samples (1 g) with ethanol (50 mL) in glass vials and left maceration for 24 h at room temperature under continuous stirring. Solutions were centrifuged ($6800 \times g/20$ min) and extraction was repeated three times. Obtained extracts were combined and stored at 4 °C until the analyses were realized.

2.4. Antioxidant components

2.4.1. Total phenolic compounds

Total phenolic compounds content was determined using Singleton and Rossi (1965) method and the results were expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW) with the use of calibration curve obtained with gallic acid ($y = 0.0015 + 0.1483x$; $r^2 = 0.999$).

2.4.2. Total flavonoids content

Flavonoids content was quantified using the method reported by Huang et al. (2004). Amounts of flavonoids were deduced from a standard curve ($y = -0.0008 + 0.1162x$; $r^2 = 0.994$) and calculated in mg quercetin equivalents (QE)/100 g dry weight (DW).

2.4.3. Flavonols content

Total flavonols in the studied samples were assayed by the method reported by Adedapo et al. (2008) and the amounts were expressed as mg of quercetin equivalents per 100 g of dry weight (mg EQ/100 g DW). Quercetin was used to establish the calibration curve ($y = -0.0003 + 2.2549x$; $r^2 = 0.999$).

2.4.4. Proanthocyanidins content

Proanthocyanidins content was determined by the method reported by Maksimović et al. (2005) using butanol-HCl and the amounts were expressed as mg (+)-catechin equivalent (CE) 100 g^{-1} DW ($y = -0.1488 + 2.078x$; $r^2 = 0.999$).

2.4.5. Ortho-diphenols content

Ortho-diphenols contents were determined by the method described by Tovar et al. (2002) and were expressed as mg equivalents of gallic acid (EGA)/100 g DW ($y = -0.0087 + 0.0848x$; $r^2 = 0.998$).

2.4.6. Ascorbic acid content

Ascorbic acid contents were determined using the method described by Mau et al. (2005) and the amounts were expressed as

mg ascorbic acid equivalents (AAE)/100 g DW ($y = 1.0142 - 18.333x$, $r^2 = 0.999$).

2.4.7. Carotenoids content

2.4.7.1. Extraction procedure. The analysis of carotenoids was performed following the protocol of Melendez-Martinez et al. (2007). Sample powders (20 g) were added with 15 mL of a mixture hexane/acetone/methanol (2/1/1, v/v/v) and were placed into ultrasonic (Selecta, Spain) for 5 min. Extraction was repeated three times. After centrifugation at 4500 rpm for 10 min (20 °C), supernatants were concentrated to dryness in rotary evaporator at 30 °C, added with 150 μL of hexane and centrifuged (14,000 × *g*/5 min/4 °C) prior to its injection in the rapid resolution liquid chromatography (RRLC).

2.4.7.2. Rapid resolution liquid chromatography conditions. Carotenoid extracts were separated and quantified by RRLC (Agilent 1260 system equipped with a diode-array detector) using polymeric C18 Poroshell 120 column (2.7 μm, 5 cm × 4.6 mm) (Agilent, Palo Alto, CA). Acetonitrile (solvent A), methanol (solvent B) and acetate ethyl (solvent C) were the mobile phase used in this analysis. The gradient elution profile was as follows: 0 min, 85% A + 15% B; between 5 and 7 min, 60% A + 20% B + 20% C; between 9 and 12 min, 85% A + 15% B. The chromatograms were monitored at 450 nm and the identification of carotenoids was made by comparison of the chromatographic and UV/VIS spectroscopic characteristics with those of standards. The carotenoid compounds were quantified based on the area under the detected peaks against calibrated standards. Total carotenoids were expressed as sum of single quantified carotenoids.

2.4.8. Color

The Vis-NIR reflectance spectra were acquired using a spectrometer Spectro-320 (Instrument Systems, Germany) coupled with an integrating sphere ISP-150 (Instruments Systems, Germany) and using a Certified Reflectance Standard (Labsphere, North Sutton NH, USA). Each sample was put into a quartz cell and was placed in the integrating sphere with diffuse illumination and an observation angle of 8° (geometry "d/8°"). The Specwin v. 1.8.1.6 software (Instruments Systems, Germany) recorded the reflectance spectrum from 380 to 1800 nm ($\Delta l = 2$ nm) as well as the colorimetric coordinates L^*, a^*, b^*, C^*_{ab}, and h_{ab} of each sample according to the International Commission on Illumination (CIE) specifications (Gordillo et al., 2015).

2.5. Antioxidant activities

2.5.1. DPPH•radical scavenging activity

The DPPH (1,1-diphenyl-2-picrilhydrazyl) radical scavenging activity exerted by the studied extracts was evaluated following the method described by Adedapo et al. (2008). Methanolic solutions of butylated hydroxyanisole (BHA), quercetin and catechin used as pure compounds were tested too. The percentage inhibition of the DPPH radical was calculated by the following equation (Eq. (1)):

$$I(\%) = [(A_{control} - A_{sample})/A_{control}] * 100 \quad (1)$$

where I (%) is the percentage scavenging of the DPPH radical, $A_{control}$ is the absorbance of the control reaction (a reaction with all the reagents except the sample extract) and A_{sample} is the absorbance of the sample extract.

2.5.2. $ABTS^+$ radical cation scavenging power

The 2,2′-azinobis(3-ethylbenzothiazoline-6-sufonic acid ($ABTS^+$) radical scavenging power was determined by the protocol described by Adedapo et al. (2008). The percentage of $ABTS^+$ inhibition was calculated using the Eq. (1).

2.5.3. Superoxide anion radical scavenging activity

Samples were tested for its capacity to scavenge superoxide anion radicals using the protocol reported by Dastmalchi et al. (2008). The absorbance of solutions was measured at 560 and 293 nm to detect if the extract could inhibit uric acid production. The percentage inhibition was calculated using Eq. (1).

2.5.4. Hydroxyl radical scavenging activity in salicylic acid system

Hydroxyl radical scavenging activity in salicylic acid system was assayed according to Gu et al. (2008). BHA, quercetin and catechin were used as positive controls. Hydroxyl radical scavenging ability was evaluated as the inhibition rate of salicylic acid oxidation by hydroxyl radical, and was calculated with Eq. (1).

2.5.5. Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was carried out using the method reported by Dastmalchi et al. (2008). The percentage of inhibition was calculated using the Eq. (1).

2.5.6. Phosphomolybdenum assay (total antioxidant activity)

Total antioxidant activity exerted by extracts was assessed with the phosphomolybdenum reduction assay following the method reported by Amessis-Ouchemoukh et al. (2014b). Total antioxidant activity was expressed as mg gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW) using the calibration curve with gallic acid ($y = -0.0027 + 4.4401x$; $r^2 = 0.999$).

2.5.7. Determination of ferric-reducing antioxidant power (FRAP)

The method develloped by Adedapo et al. (2008) was applied to determine the ferric-reducing antioxidant power of samples. The change in absorbance from the red to the blue was followed at 593 nm. A calibration curve was done with catechin ($y = 0.6654 + 17.06x$; $r^2 = 0.930$) and the obtained results were expressed in mg catechin equivalents per 100 g dry weight (mg CE/100 g DW).

2.5.8. Reducing power

The reducing power of the extracts was determined by the method used by Gülçin et al. (2004). The absorbance was measured at 700 nm.

2.5.9. Metal chelating activity

The capacity of extracts to chelate iron (II) was carried out by the protocol developed by Gülçin et al. (2004). Percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated with Eq. (1).

2.6. Hydrogen peroxide scavenging activity

Scavenging of hydrogen peroxide was assayed with the method reported by Gülçin et al. (2004). Extract samples or positive controls (BHA, catechin and quercetin) were tested and the absorbance of hydrogen peroxide was determined at 230 nm. The percentage of hydrogen peroxide scavenging activity was calculated according to Eq. (1).

2.7. Anti-inflammatory activity: xanthine oxidase inhibition assay

The xanthine oxidase (XO) inhibitory activity was conducted using the method described by Havlik et al. (2010). Absorbance was measured at 295 nm and allopurinol was assayed as a positive

control. Xanthine oxidase inhibition activity was calculated using the following Equation:

$$\%\text{inhibition} = [1 - (A_1/A_0)] * 100 \quad (2)$$

Where A_1 and A_0 are the activities of the enzyme in the presence and the absence of extract or positive control, respectively.

2.8. Calpain activity, reactive oxygen species (ROS) and cytotoxic effect on human glioblastoma (U87) cells

2.8.1. U87 culture cell

Human glioblastoma U87 cells were cultured in Eagle's minimal essential medium supplemented with penicillin (50 IU), fetal calf serum (10%) and streptomycin (50 μg/mL). Glioblastoma cells were maintained at 37 °C in a humidified atmosphere (5% CO_2).

2.8.2. Reactive oxygen species (ROS) production

ROS production was determined using the chemiluminescence of lucigenin on a 96-well microplate (Greiner Cellstar) following the method of Simões et al. (2004). Human glioblastoma (U87) cells were starved of fetal calf serum for 48 h and seeded at a density of 20000 cells per well in 96 well plates. Plates were treated with (25 μg/mL, 100 μg/mL, 250 μg/mL) and incubated for three different time intervals: 15, 30 and 60 min. A control containing only cells was prepared in each plate together with nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor diphenylene iodonium (DPI, 0.01 mM and 0.2 mM). After each incubation time, 5 μL of a mixed solution containing lucigenin (6 mM) and NADPH (4 mM) prepared in a blank medium were added. Luminescence at 490 and 538 nm for excitation and emission, respectively was determined immediately at 37 °C by a Fluoroscan Ascent FL fluorimeter (Labsystems, France). Results were obtained as the integration of the signal assessed each minute for 45 min of measurement. The percentage of lucigenin chemiluminescence inhibition was calculated with Eq. (1).

2.8.3. Anti-calpain activity

The anti-calpain activity exerted by samples was assessed following the method of Rosser et al. (1993) that is based on the fluorogenic calpain substrate *tert*-butoxycarbonyl-Leu-Met-chloromethylaminocoumarin (t-boc-LM-CMAC). This substrate is a coumarin derivative with a maximum excitation/emission at 330/403 nm. Its cleavage by calpains causes an increase in the maximum excitation/emission at 351/430 nm. In 96-well black plates previously treated for 1 h with collagen (10 μg/mL), cells were seeded (20000 cells per well). After 24 h, cells were treated with three different concentrations of extracts (25, 100 and 250 μg/mL) for varying incubation times (2, 4 and 6 h). After incubation with t-boc-LM-CMAC (25 mM) for 25 min, cells were washed with Hank's medium Balanced Salt Solution (HBSS) without phenol red. Reading was done using a Fluoroskan with an excitation wavelength of 351 nm and an emission wavelength of 430 nm. Results obtained in relative fluorescence units (RFU) were compared with the control and expressed in percentage inhibition of calpain activity.

2.8.4. Cytotoxicity assay

The MTT assay described by Mossman (1983) was used to assess the antiproliferative effects of the studied extracts. Exponentially growing cells were distributed over 96-wells microplates at a density of 5000 cells per well, in 100 μL of EMEM culture medium. After 2 h of incubation at 37 °C, extracts were diluted in phosphate-buffered saline and were added to each well and incubation was pursued for 72 h. Then, 0.5 mg/mL of MTT were added in the complete medium for 2 h at 37 °C. Plates were washed with phosphate-buffered saline (100 μL) and the formazan crystals were solubilized with 100 μL dimethyl sulfoxide (DMSO) to determine the absorbance at 600 nm.

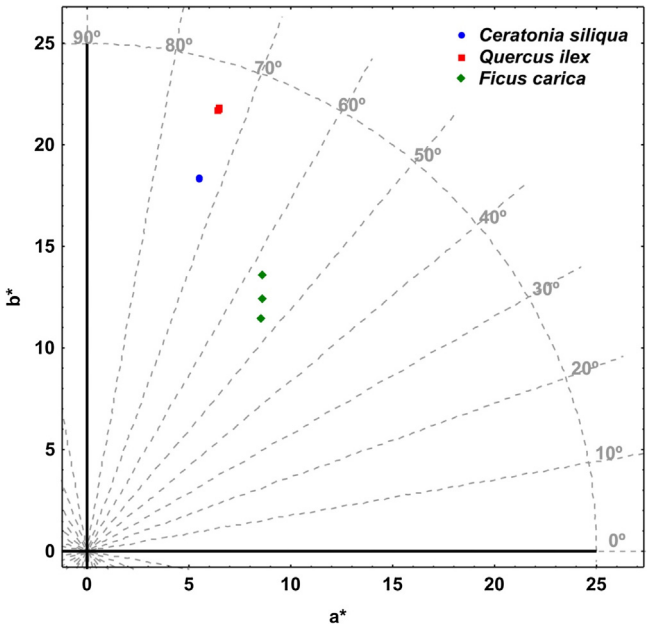

Fig. 1. Location of the samples in the a*b* plane and L* scale. Spectroradiometry measurements.

3. Statistical analysis

All the experiments were done in triplicates and the results were given as mean ± standard deviation (*S.D.*). One-way analysis of variance (*ANOVA*) with a *post hoc* least significant difference test was used to determine significance ($^*p \leq 0.05$) with STATISTICA 5.5 software together with correlation matrix with the use of elementary statistics.

4. Results and discussion

4.1. Determination of the amounts of antioxidants

In this study, several phenolic groups were quantified. Among analyzed extracts, extremely high total contents of phenolics, flavonoids and *ortho*-diphenols were detected in *Quercus ilex* (Table 1). This later contained seven times more phenolics and three times more flavonoids than *Ceratonia siliqua*. The lowest total phenolics, flavonoids and *ortho*-diphenols levels were found in *Ficus carica*. The amounts of flavonols were similar in *Quercus ilex* and *Ficus carica* while proanthocyanidins contents were the highest in *Ficus carica*. Several works found that *Ceratonia siliqua* and *Ficus carica* were rich in phenolic compounds, flavonoids and flavonols and the obtained results agreed with them (Ayaz et al., 2007; Çalişkan and Aytekin Polat, 2011; Roseiro et al., 2013a. However *Quercus ilex*, in addition to the above reported compounds, contained tanins, and polyacylated flavonoid glucosides (Cantos et al., 2003; Karioti et al., 2010; Custódio et al., 2013). Another important substance investigated in this study was ascorbic acid (Table 1). *Ceratonia siliqua* and *Quercus ilex* had the highest amounts and were higher than those found by Gubbuk et al. (2010) in the study about carob (8.07–10.41 mg/100 g). Variation in the amounts of antioxidants could be attributed to several reasons like the solubility of phenolic compounds that are directly related the type of solvent utilized and the interaction of these compounds with other components in

Table 1
Total phenolics, flavonoids, flavonols, proanthocyanidins, *ortho*-diphenols and ascorbic acid contents in dry extracts.

	Total phenolics (mg CE/100 g DW)	Flavonoids (mg QE/100 g DW)	Flavonols (mg QE/100 g DW)	Proanthocyanidins (mg CE/100 g DW)	Ortho-diphenols (mg GAE/100 g DW)	Ascorbic acid (mg AAE/100 g DW)
Ficus carica	2190.10 ± 17.55^{c}	858.43 ± 10.76^{c}	19.57 ± 0.55^{b}	496.63 ± 3.61^{a}	1189.56 ± 19.00^{c}	14.21 ± 0.07^{c}
Ceratonia siliqua	5165.49 ± 38.32^{b}	1342.51 ± 28.46^{b}	38.23 ± 0.64^{a}	140.00 ± 5.47^{c}	5272.70 ± 24.25^{b}	36.29 ± 0.49^{a}
Quercus ilex	37908.80 ± 34.97^{a}	3863.310 ± 25.60^{a}	20.308 ± 0.32^{b}	254.53 ± 2.78^{b}	7734.87 ± 25.03^{a}	35.56 ± 0.26^{b}

Averages ± Standard Deviation were obtained from three different experiments.
Means having different letter are significantly different.
CE: Catechin Equivalents.
QE: Quercetin Equivalents.
GAE: Gallic Acid Equivalents.
AAE: Ascorbic Acid Equivalents.
DW: Dry Weight.

extracts leading to the formation of insoluble complex (Amessis-Ouchemoukh et al., 2014a).

4.2. Carotenoids content

Several carotenoids were identified in the studied matrix (Table 2). *Ceratonia siliqua* pods showed more carotenoids than other samples and pheophytin and its derivatives were dominants. Abdul Karim and Azlan (2012) have reported the presence of carotenoids in *Ceratonia siliqua* pods like α-carotene, lycopene, lutein and β-carotene. The last compound was also identified in the studied sample. Three carotenoids were identified in *Ficus carica,* β-cryptoxanthin, α-carotene derivate and β-carotene. Literature is full of work relating the presence of carotenoids in figs (Yemiş et al., 2012; Soltana et al., 2016). In this work, carotenoids profile of *Quercus ilex* kernels was determined for the first time and three compounds were detected: β-cryptoxanthin derivate, β-carotene and α-carotene. The β-carotene was detected in three samples and its content varied between 2.08 ± 0.08 and 4.32 ± 0.14 μg/100 g. Rodriguez-Bernaldo de Quiros and Costa (2006) reported that carotenoid content in vegetables is influenced by the cultivar/variety, sample ripeness, climate, season and geographic site of production, part of plant utilized, processing and storage conditions and the type of soil.

4.3. Color

The colorimetric parameters of the samples studied were summarized in Table 3. *Ceratonia siliqua* was characterized by the highest value of lightness (L^*), followed by *Quercus ilex* and *Ficus carica*. A higher value of chroma (C^*_{ab}) was observed in *Quercus ilex* extract. The tested samples presented significant differences for all the different CIELAB color parameters, except h_{ab} that was not different between *Ceratonia siliqua* and *Quercus ilex* ($p < 0.05$). L^* and C^*_{ab} values obtained in this study were higher than those found by Çalişkan and Aytekin Polat (2011) about black figs from Turkey, 27.8 ± 6.5 and 13.5 ± 4.4 units CIELAB, respectively.

The colorimetric parameters were graphically depicted in the color diagram (Fig. 1). This later indicated the location of the color distribution on the CIELAB color space. It could be pointed out that all the tested samples were located in the first quadrant (positive values of a^* and b^*) of the (a^*b^*)-plane. The points corresponding to *Ficus carica* were more scattered than those belonging to *Ceratonia siliqua* and *Quercus ilex*. Differences in colorimetric values were attributed to the variability of the pigments composition like carotenoids and flavonoids of each sample which deeply affects its color characteristics. It was reported that pigments change the color parameters values in terms of light absorption (Fernández-Lara et al., 2015).

4.4. Determination of antioxidant capacities

Antioxidant activities (Fig. 2a, b) showed that *Quercus ilex* and *Ceratonia siliqua* were very effective in scavenging DPPH and ABTS radicals, 93.93 ± 0.13 and $82.45 \pm 0.23\%$ in DPPH assay and 83.09 ± 0.07 and $81.51 \pm 0.12\%$ in ABTS assay, respectively. Obtained inhibitions were better than those displayed by standards BHA, catechin, quercetin and trolox with 26.63 ± 0.56, 56.09 ± 0.24, 70.43 ± 0.15 and $61.21 \pm 1.15\%$, respectively. *Ficus carica* had lower efficacy to scavenge DPPH ($20.54 \pm 0.30\%$) than ABTS radicals ($68.98 \pm 0.12\%$). These results were in agreement with those reported by several studies about these samples (Vijaya Kumar Reddy et al., 2010; Custódio et al., 2013; Roseiro et al., 2013a; Feng et al., 2015). All the tested samples showed moderate effects on superoxyde anion scavenging activity (Fig. 2c). The best hydroxyl radical scavenging activity was displayed by

Table 2
Carotenoid compounds in crops analyzed.

Matrix	Retention time (min)	Identified Carotenoids	Mean ± S.D. (μg/100 g DW)
Ficus carica	3.01	β-Cryptoxanthin	2.14 ± 0.03
	7.19	β-Carotene	4.32 ± 0.14
	8.24	α-Carotene derivative	–
Ceratonia siliqua	1.00	Pheophytin a derivative 1	–
	2.35	Chlorophyll b	4.53 ± 2.18
	4	Chlorophyll a	–
	4.4	Pheophytin b2 derivative 1	–
	4.68	Pheophytin b2 derivative 2	–
	4.91	Pheophytin b1 derivative 1	–
	4.97	Pheophytin b1	–
	5.25	Pheophytin b2	–
	6.40	Pheophytin a derivative 2	–
	6.61	Pheophytin a	–
	7.33	β-Carotene	2.08 ± 0.08
	7.99	α-Carotene derivative	–
Quercus ilex	6.10	β-Cryptoxanthin derivative	–
	7.21	β-Carotene	3.53 ± 0.00
	8.36	α-Carotene	2.87 ± 0.40

Table 3
CIELAB color parameters of *Ceratonia siliqua, Ficus carica* and *Quercus ilex* studied.

Samples	L^*	a^*	b^*	C^*_{ab}	h_{ab}
Ceratonia siliqua	71.85 ± 0.03^a	5.47 ± 0.02^c	18.38 ± 0.04^b	19.17 ± 0.05^b	73.44 ± 0.02^a
Ficus carica	42.34 ± 0.42^c	8.29 ± 0.24^a	11.81 ± 0.44^c	14.43 ± 0.26^c	54.92 ± 1.68^b
Quercus ilex	66.01 ± 0.06^b	6.42 ± 0.01^b	21.79 ± 0.04^a	22.71 ± 0.04^a	73.57 ± 0.03^a

Different letters within the same column mean that there are significant differences ($p < 0.05$).

Table 4
Correlation matrix between the different studied parameters.

	TPC	Flav	Flavo	Proanth	Orthdi	AsA	MTT	RP	ABTS	DPPH	FRAP	MCP	H_2O_2	OH	NO	TAA	O_2	XO	ROS	t-BOC
TPC	1.00***	−0.40	−0.27	0.83**	0.54	−0.71*	1.00***	0.64	0.68*	0.70*	0.60	0.93***	0.75*	−0.08	−0.73*	−0.68*	0.97***	0.77*	−0.86**	
Flav		−0.33	−0.34	0.87**	0.60	−0.75*	0.99***	0.70*	0.73*	0.76*	0.54	0.90***	0.70*	−0.00	−0.78*	−0.62	0.95***	0.80*	−0.86**	
Flavo			−0.76*	0.18	0.55	−0.36	−0,45	0.44	0.40	0.37	−0.97***	−0.70*	−0.90***	0.70*	−0.33	0.80**	−0.61	0.12	0.28	
Proanth				−0.76*	−0.94***	0.85**	−0,22	−0.90***	−0.88*	−0.86**	0.59	0.08	0.42	−0.69*	0.85**	−0.34	−0.04	−0.65	0.33	
Orthdi					0.92***	−0.97***	0.80**	0.96***	0.97***	0.98***	0.05	0.58	0.26	0.34	−0.99***	−0.24	0.67*	0.90***	−0.75*	
AsA						−0.97***	0.50	0.99***	0.98***	0.98***	−0.35	0.20	−0.15	0.56	−0.97***	0.12	0.32	0.80**	−0.52	
MTT							−0.67*	−0.99***	−0.99***	−0.99***	0.13	−0.41	−0.07	−0.45	0.99***	0.06	−0.52	0.60	0.45	
RP								0.61	0.64	0.67*	0.64	1.00	0.79*	−0.12	−0.70*	−0.70*	0.98***	0.75*	−0.85**	
ABTS									1.00***	1.00*	1.00	0.33	−0.55	0.51	−0.99***	0.00	0.44	0.85**	−0.61	
DPPH										1.00***	−0.18	0.37	0.03	0.48	−1.0***	−0.04	0.48	0.86**	−0.64	
FRAP											−0 0.14	0.41	0.06	0.46	−1.0***	−0.07	0.51	0.87**	−0.66	
MCP												0.85**	0.98***	−0.63	0.10	−0.86**	0.77*	0.10	−0 0.46	
H_2O_2													0.94***	−0.34	−0.44	−0.84**	0.99***	0.56	−0.87*	
OH														−0.55	−0.10	−0.88**	0.89**	0.29	−0.59	
NO															−0.44	0.46	−0.27	0.07	−0.20	
TAA																0.11	−0.55	−0.88	0.68*	
O_2																	−0.78*	−0.19	0.68*	
XO																		0.65	−0.81**	
ROS																			−0.53	
t-BOC																				

Abbreviations: TPC: Total phenolic compounds.
Flav: Flavonoids.
Flavo: Flavonols.
Proanth: Proanthocyanidins.
Orthdi: Ortho diphenols.
AsA: Ascorbic acid.
MTT: MTT assay.
RP: Reducing Power.
ABTS: 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid.
DPPH: 1,1-diphenyl-2-picrilhydrazyl radical.
FRAP: Ferric Reducing Antioxidant Power.
MCP: Metal Chelating Power.
H_2O_2: Hydrogen peroxide.
OH: hydroxyl radical.
NO: Nitric Oxide.
TAA: Total Antioxidant Activity.
O_2: Superoxide anion.
XO: Xanthine Oxidase.
ROS: Reactive Oxygen species.
t-BOC: *tert*-butoxycarbonyl.
Significance of correlations: $*p < 0.05$: Significant correlation; $**$ $p < 0.01$: Very significant correlation; $***p < 0.001$: Extremely significant correlation.

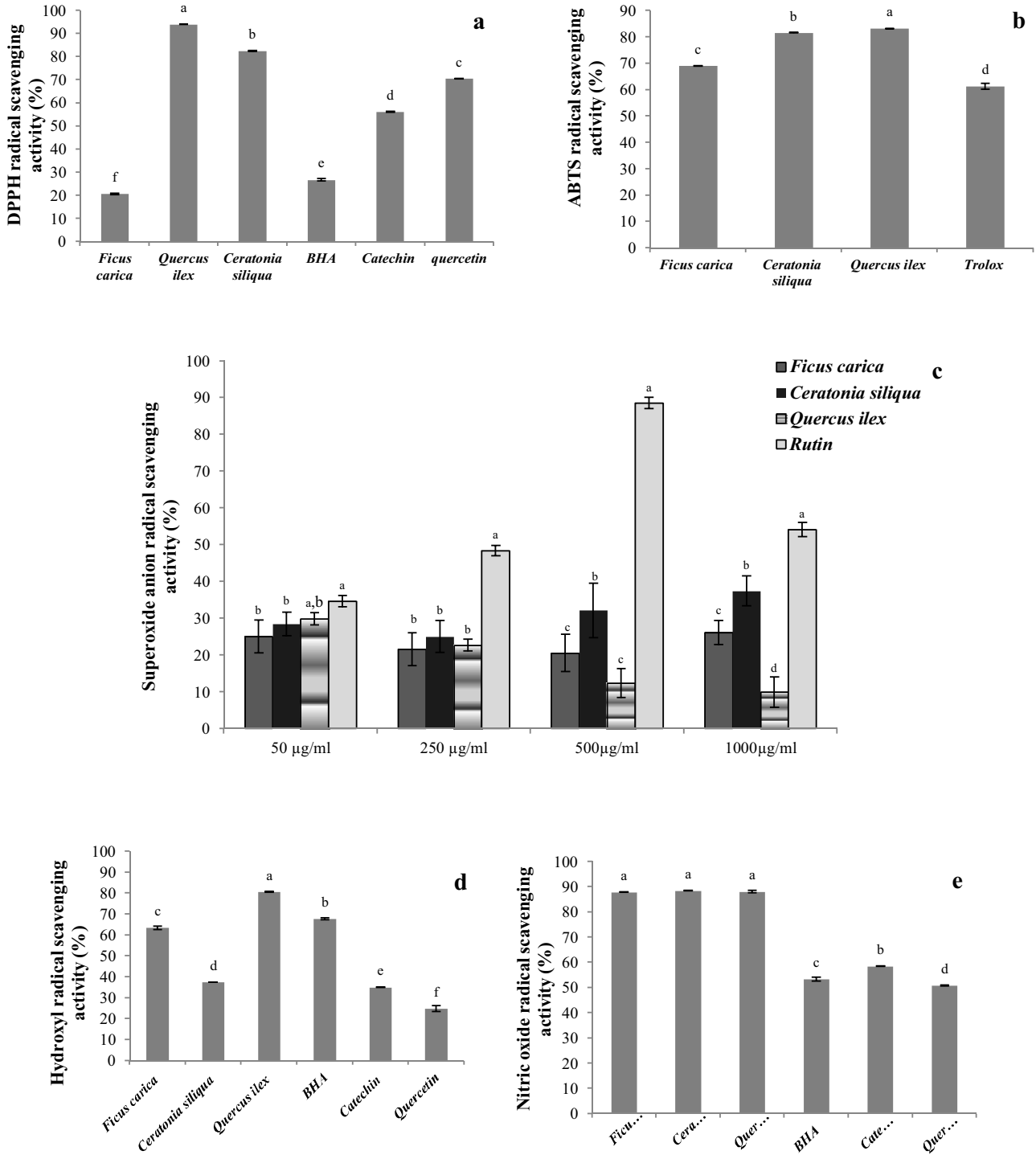

Fig. 2. Antioxidant activities presented by samples and standards towards different radical species: a: DPPH radical scavenging activity; b: ABTS radical scavenging activity; c: Superoxide anion radical scavenging activity; d: Hydroxyl radical scavenging activity; e: Nitric oxide radical scavenging activity. Different letter(s) indicate the values are significantly different (*$p < 0.05$).

Quercus ilex with 80.51 ± 0.20%, hence exceeding that found with BHA, catechin and quercetin with 67.64 ± 0.53, 34.92 ± 0.13 and 24.69 ± 1.38%, respectively (Fig. 2d). Nitric oxide radical scavenging activities (Fig. 2e) showed no significant differences between *Ceratonia siliqua*, *Quercus ilex* and *Ficus carica*, 88.35 ± 0.11, 87.99 ± 0.41 and 87.81 ± 0.11%, respectively. The obtained inhibitions were very high compared with catechin (58.36 ± 0.11%), BHA (53.22 ± 0.76%) and quercetin (50.81 ± 0.19%). All extracts exhibited reducing powers (Fig. 3) which is in accordance with several results found in the literature (Çalişkan and Aytekin Polat, 2011; Custódio et al., 2013; Sęczyk et al., 2016). In phosphomolybdenum assay, *Ficus carica* presented a higher reducing ability with 638.23 ± 0.43 mg GAE/100 g (Fig. 3a), while *Quercus ilex* was very potent in FRAP (Fig. 3b) and reducing power assays (Fig. 3c), with 114.79 ± 0.063 mg CE/100 g and 1.513 ± 0.00 at 0.08 mg/mL, respectively. In accordance with FRAP test, several works reported the reducing power of *Ficus*

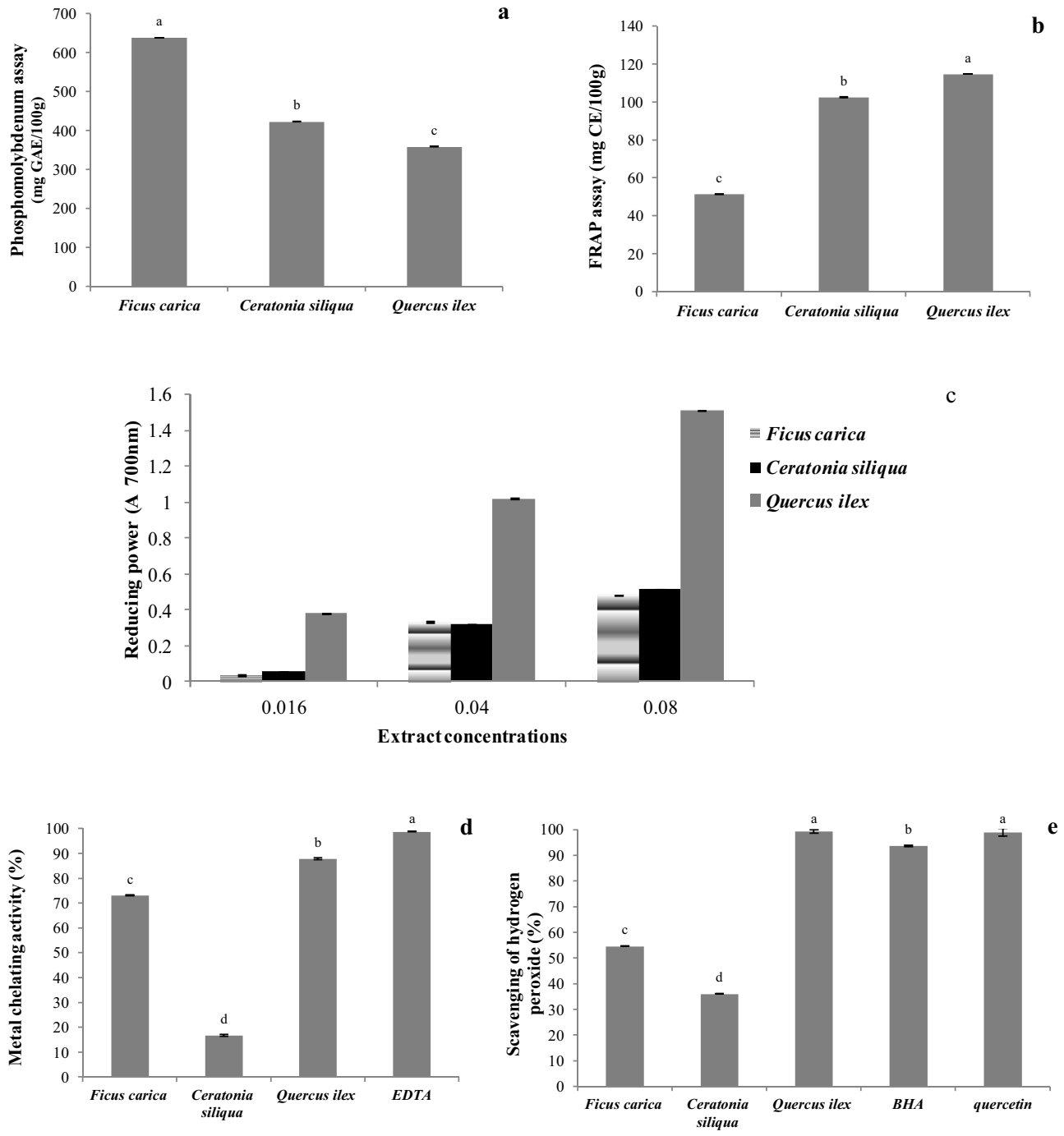

Fig. 3. Power reduction, neutralization and chelation presented by dry extracts: a: Phosphomolybdenum assay; b: FRAP assay; c: Reducing power; d: Metal chelating activity; e: Scavenging of hydrogen peroxide. Different letter(s) indicate the values are significantly different ($^{*}p < 0.05$).

carica (Feng et al., 2015; Vijaya Kumar Reddy et al., 2010) and *Ceratonia siliqua* (Vitali Čepo et al., 2014). *Quercus ilex* and *Ficus carica* extracts inhibited the formation of complex Fe^{2+}-ferrozine (Fig. 3d). Metal chelating power of these samples were 87.87 ± 0.34 and $73.17 \pm 0.16\%$, respectively. These two crops were also able to scavenge H_2O_2 efficiently (Fig. 3e). *Quercus ilex* presented the best activity ($99.33 \pm 0.63\%$) together with the standard quercetin ($99.06 \pm 1.50\%$). From the present results, studied samples showed differences in antioxidant activities that could be related to the anti-radical components that are extracted from the different extracts. Several correlation coefficients were calculated between phenolic compounds and DPPH ($r = 0.68^{*}$), FRAP ($r = 0.70^{*}$), H_2O_2 ($r = 0.93^{***}$) OH ($r = 0.75^{*}$) and reducing power ($r = 1.00^{***}$) (Table 4). Flavonoids also showed correlations with ABTS ($r = 0.70^{*}$), DPPH ($r = 0.73^{*}$) and reducing power ($r = 0.99^{***}$). These correlations confirmed the involvement and the role of phenolic compounds in the antioxidant activities.

4.5. Xanthine oxidase inhibition assay

Determination of XO inhibitory activity is based on the measure of uric acid production from xanthine or hypoxanthine

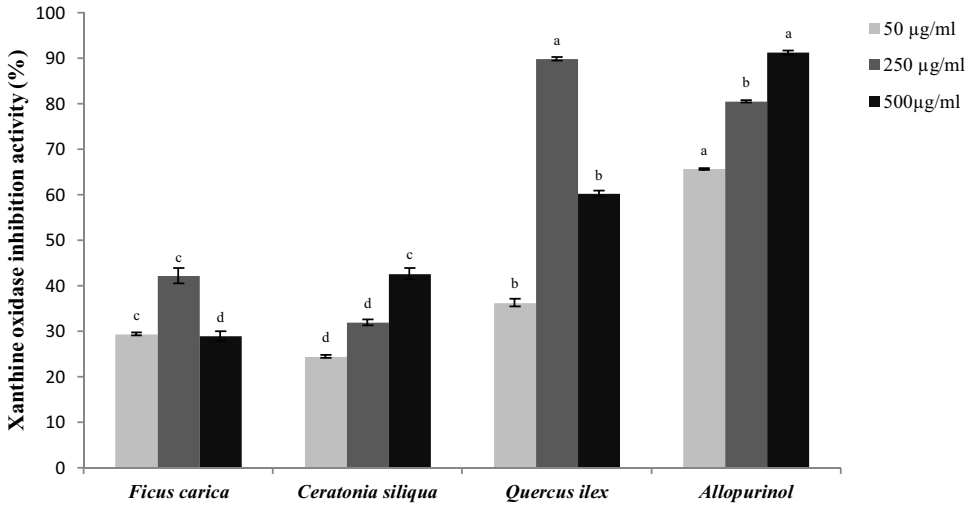

Fig. 4. Xanthine oxidase inhibitory activity of sample extracts and allopurinol. Data were analyzed using One-Way *ANOVA* (Analysis Of Variance). For each concentration, different letter(s) indicate the values are significantly different ($^{*}p < 0.05$).

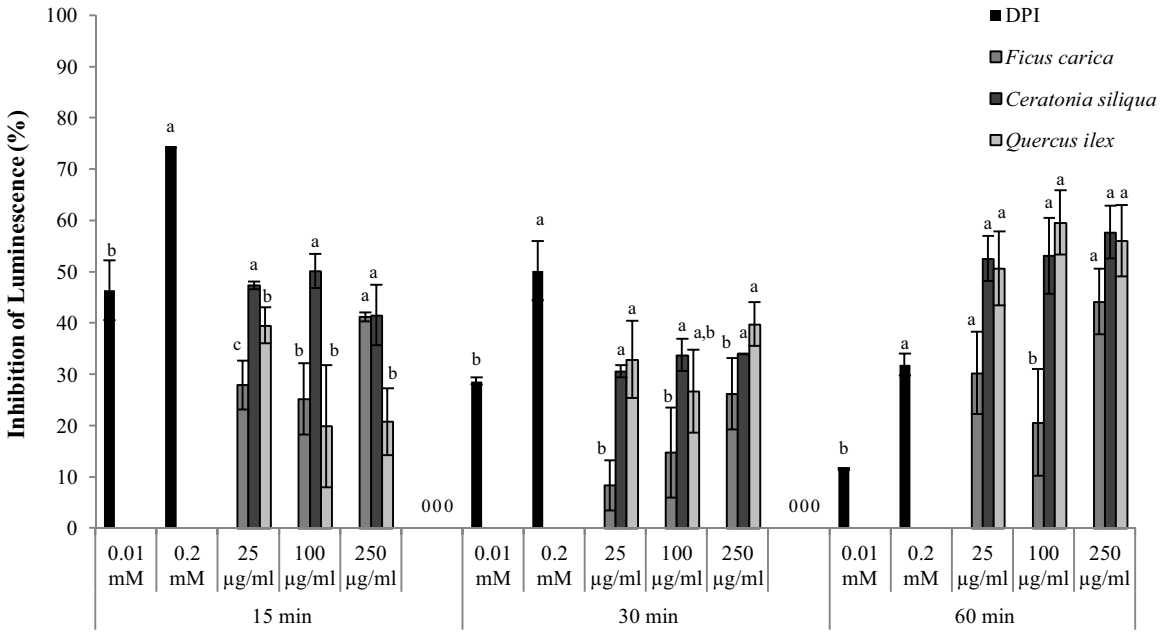

Fig. 5. Inhibition of chemiluminescence of lucigenin by samples. Data were analyzed using One-Way *ANOVA*. For each concentration and incubation time, different letter(s) indicate the values are significantly different ($^{*}p < 0.05$).

substrate (Lin et al., 2002). As shown in Fig. 4, all the samples were able to inhibit the activity of the enzyme XO. At 50 µg/mL, the best activity was obtained with *Quercus ilex* (36.24 ± 0.84%), followed by *Ficus carica* (29.38 ± 0.32%) and *Ceratonia siliqua* (24.46 ± 0.32%). At 250 µg/mL, *Quercus ilex* presented the best inhibition, 89.81 ± 0.36%, exceeding that of the positive control allopurinol (80.52 ± 0.24%), a drug clinically used for gout treatment (Havlik et al., 2010). This later together with *Ceratonia siliqua* extract inhibited XO activity in a concentration-dependent manner, whereas *Quercus ilex* and *Ficus carica* extracts showed a decrease in the inhibition of XO activity when tested at 500 µg/mL as the result of its pro-oxidant effect. It was reported that phenolic compounds, flavonoids and tanins strongly inhibit the activity of XO (Bhouri et al., 2010) and these compounds were found in the tested extracts. In addition, correlation coefficients were obtained between XO inhibition activity and total phenolics ($r = 0.97^{***}$), flavonoids ($r = 0.95^{***}$) and *ortho*-diphenols ($r = 0.67^{*}$) (Table 4). The obtained results were in accordance with several correlations found with these compounds in the literature. Moreover, it was shown that there are structural and electronic similarities of purine ring of xanthine and A ring of flavonoids (Lin et al., 2002; Havlik et al., 2010).

4.6. ROS production

The amounts of ROS produced in the presence of samples was measured by chemiluminescence using lucigenin. This method is widely used to determine the rate of superoxide radicals in human neutrophils (Hasegawa et al., 1997; Aam and Fonnum, 2007). From the obtained results (Fig. 5), all the samples inhibited the chemiluminescence of lucigenin and ROS production and differed from each other according to the concentration of the sample and the incubation time. After 15 min of treatment, *Ceratonia siliqua* presented the best capacity to ROS inhibition with 47.32 ± 0.73, 50.16 ± 3.33

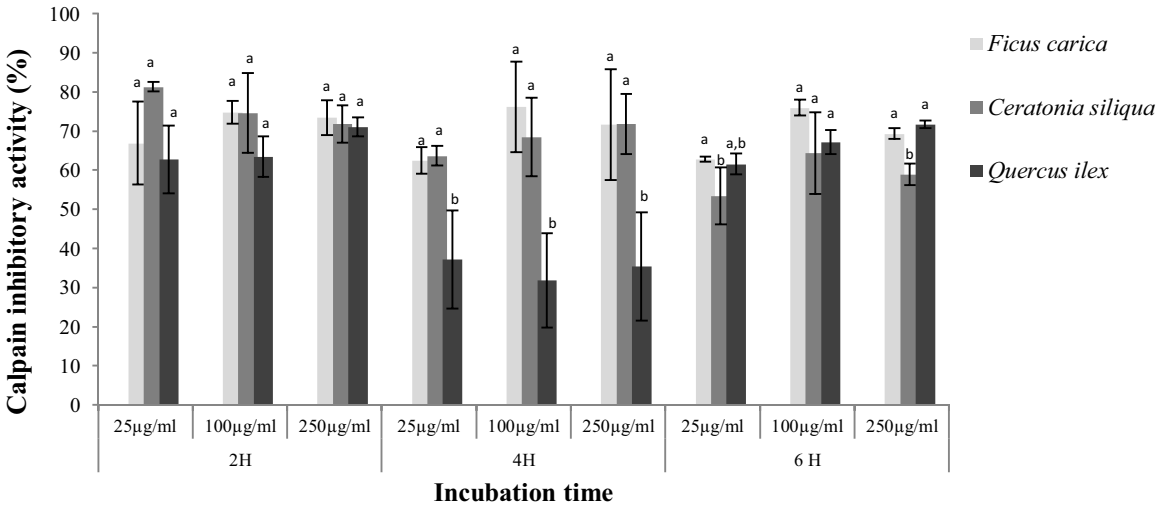

Fig. 6. Calpain activity in U87 cells untreated or treated with extracts of samples. The activity measured in RFU is expressed as a percentage. Data were analyzed using One-Way *ANOVA* (Analysis Of Variance). For each concentration and incubation time, different letter(s) indicate the values are significantly different (*$p < 0.05$).

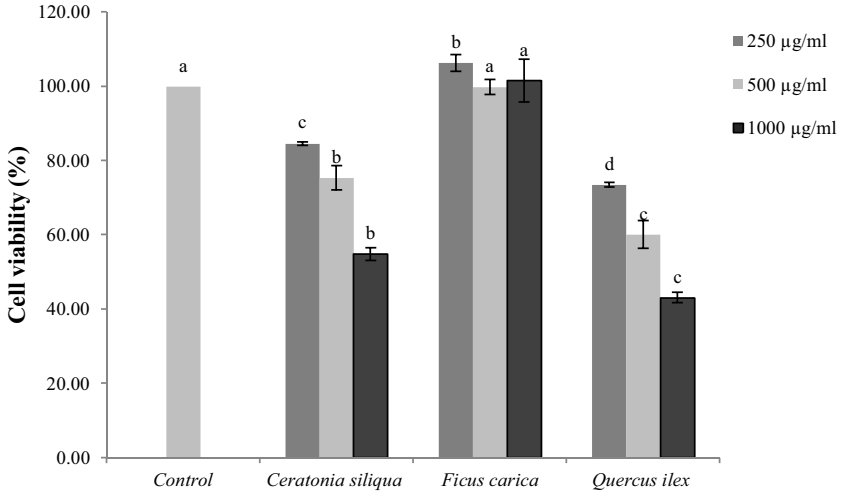

Fig. 7. Effect of dry extracts on the survival of U87 glioblastoma cells. Data were analyzed using One-Way *ANOVA* (Analysis Of Variance). For each concentration different letter(s) indicate the values are significantly different (*$p < 0.05$).

and 41.53 ± 5.89% for 25, 100 and 250 µg/mL, respectively. This was followed with *Quercus ilex* that showed 39.51 ± 3.50% of ROS inhibition when tested at 25 µg/mL. *Ficus carica* seemed to reach its higher level of inhibition 41.21 ± 0.88% at 250 µg/mL. The standard DPI, a selective inhibitor of NADPH oxidase (Tazzeo et al., 2009), tested at 0.2 mM showed 74.17 ± 0.04% of lucigenin inhibition.

After 30 min of incubation time, the chemiluminescence of lucigenin decreased in all the tested samples and the standard DPI, exception done for *Quercus ilex* where a moderate augmentation was observed at 100 and 250 µg/mL. The decrease in inhibition of ROS generation could be attributed to the exhaustion of medium in antioxidants or a pro-oxidant effect that triggered the generation of different ROS. In addition, glioblastoma cells could resume its activity by developing resistance and increasing production of ROS. This case has already been discussed in the literature by Pelicano et al. (2004). The inhibition exerted by DPI was still decreasing after 6 h of treatment while an increase was observed in dry extracts due to the reduction in cell viability.

Ceratonia siliqua and *Quercus ilex* extras presented the best capacities of ROS inhibition. These findings could be attributed to its phenolic contents. Colorimetric dosage had demonstrated that these samples were rich in these compounds and correlation coefficients were obtained between ROS inhibition and total phenolics ($r = 0.77^{*}$), flavonoids ($r = 0.80^{*}$), flavonols ($r = 0.90^{***}$) and *ortho*-diphenols ($r = 0.80^{**}$) (Table 4). In addition, high correlations were obtained between the inhibition of chemiluminescence of lucigenin and antioxidant activity assays: reducing power ($r = 0.75^{*}$), ABTS radical scavenging assay ($r = 0.85^{**}$), DPPH radical scavenging assay ($r = 0.86^{**}$) and FRAP assay ($r = 0.87^{**}$) (Table 4). The involvement of phenolic compounds in the ROS scavenger effect was previously reported by several authors using chemiluminescence of lucigenin. In fact, these compounds can act by two mechanisms: first, by inhibiting the enzyme NADPH oxidase and thus reducing the number of radicals formed and secondly by direct scavenging of superoxide radicals in competition with lucigenin (Simões et al., 2004; Franck et al., 2013).

4.7. Calpain activity

Calpain activity was examined after treatment of cells with dry extracts. The Fig. 6 showed that samples decreased the fluorescence of BOC-LM-CMAC and consequently inhibited the activity of calpain. After 2 h of treatment, the percentage of inhibition of calpain activity exceeded 50% for all the concentrations tested,

ranging from 62.76 ± 5.64 to 81.33 ± 1.26%. *Ficus carica*, *Ceratonia siliqua* and *Quercus ilex* appeared to have the same capacity to inhibit calpain and statistical analysis showed no significant differences between them. After 4 h of incubation, inhibition decreased when cells were treated with *Quercus ilex* extract. This later presented at 25, 100 and 250 μg/mL, 37.20 ± 4.57, 31.85 ± 4.02 and 35.45 ± 3.84% of calpain activity, respectively. The incubation time had no effect on the inhibitory activity of calpain in the presence of *Ficus carica* and *Ceratonia siliqua* extracts. These inhibitory activity of the studied extracts could be attributed to its chemical composition that contain several antioxidants groups especially phenolic compounds. Kim et al. (2009) have thus demonstrated that some flavonoids and flavonols like −epicatechin 5-gallate and kaempferol, exhibited inhibitory activities against calpains. These results are in accordance with the obtained results since these phenolic groups were found to be present in this extract. These flavonoids were not only able to inhibit calpain activity but were found to have antioxidant activities (Bhouri et al., 2010). In this study, correlation matrix showed significant correlation ($^{*}p < 0.05$) between calpain activity and total antioxidant activity (r = 0.68) and superoxide anion scavenging activity (r = 0.68) (Table 4).

4.8. Cytotoxicity assay

The Fig. 7 showed that *Quercus ilex* and *Ceratonia siliqua* reduced cell viability in a concentration-dependant manner, while *Ficus carica* showed no effect at the concentrations tested. Percentages of cell survival obtained in the presence of *Quercus ilex* extracts were 73.48 ± 0.64, 60.02 ± 3.74 and 43.06 ± 1.41 at 250, 500 and 1000 μg/mL. These later were the best obtained antiproliferative effects. Cytotoxic activity of *Quercus* species was previously reported (Sohretoglu et al., 2012; Lloki Assanga et al., 2013). *Ceratonia siliqua* reduced cell viability at 54.78 ± 1.72% when tested at 1000 μg/mL. It was reported that carob pods extracts contained gallic acid and gallotanins and these compounds were able to inhibit the proliferation of neuroblastoma, adenoma, adenocarcinoma and breast cancer cells (Corsi et al., 2002; Roseiro et al., 2013b). In this study, a very significant correlation was obtained between cytotoxicity assay and proanthocyanidins ($r = 0.85^{**}$) (Table 4).

5. Conclusion

From the present work, it is interesting to note that among studied samples, the highest amounts of phenolic compounds, carotenoids and ascorbic acid were detected in *Quercus ilex* and *Ceratonia siliqua*. These findings may confirm the interesting potential of the studied extracts as a valuable tank for bioactive molecules. Antioxidant activity demonstrated by the tested samples during the various used assays proves that its extracts are an excellent source of antioxidants that can serve the industries and could be exploited in pharmaceutic and cosmetic fields. *Quercus ilex* presented the best inhibition of XO exceeding that of the positive control allopurinol that is used for gout treatment. *Quercus ilex* extract, might hence be a promising alternative to synthetic substances. This sample also exerted a high antiproliferative activity on U87 glioblastoma cells, thus it could be an excellent source of antiproliferative compounds. Sample extracts were able to inhibit calpain activity, the overactivation of this protease is responsible in the appearance of several pathologies, hence, inhibitors of calpain from samples could be useful therapeutic agents for the treatment of several diseases (Kim et al., 2009). On the whole, it is interesting to note that the obtained results are useful to further research on natural products with antioxidant and chemotherapeutic attributes.

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References

Çalişkan, O., Aytekin Polat, A., 2011. Phytochemical and antioxidant properties of selected fig (*Ficus carica* L:) accessions from the Eastern Mediterranean region of Turkey. Sci. Hortic. 128, 473–478.

Aam, B.B., Fonnum, F., 2007. ROS scavenging effects of organic extract of diesel exhaust particles on human neutrophil granulocytes and rat alveolar macrophages. Toxicology 230, 207–218.

Abdul Karim, A., Azlan, A., 2012. Fruit pod extracts as a source of nutraceuticals and pharmaceuticals. Molecules 17, 11931–11946.

Adedapo, A.A., Jimoh, F.O., Koduru, S., Afolayan, A.J., Masika, P.J., 2008. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. BMC Complement. Altern. Med. 8 (53), 1–8.

Amessis-Ouchemoukh, N., Abu-Reidah, I.M., Quirantes-Piné, R., Madani, K., Segura-Carretero, A., 2014a. Phytochemical profiling, *in vitro* evaluation of total phenolic contents and antioxidant properties of *Marrubium vulgare* (horehound) leaves of plants growing in Algeria. Ind. Crops Prod. 61, 120–129.

Amessis-Ouchemoukh, N., Madani, K., Falé, L.V.P., Serralheiro, M.L., Araújo, M.E.M., 2014b. Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities. Ind. Crops Prod. 53, 6–15.

Ayaz, F.A., Torun, H., Ayaz, S., Correia, P.J., Alaiz, M., Sanz, C., Grúz, J., Strnad, M., 2007. Determination of chemical composition of anatolian carob pod (*Ceratonia siliqua* l.): sugars, amino and organic acids, minerals and phenolic compounds. J. Food Qual. 30, 1040–1055.

Bhouri, W., Derbel, S., Skandrani, I., Boubaker, J., Bouhlel, I., Sghaier, M.B., Kilani, S., Mariotte, A.M., Dijoux-Franca, M.G., Ghedira, K., Chekir-Ghedira, L., 2010. Study of genotoxic, antigenotoxic and antioxidant activities of the digallic acid isolated from *Pistacia lentiscus* fruits. Toxicol. In Vitro 24, 509–515.

Cantos, E., Espin, J., Carlos Lopez-Bote, C., De La Hoz, L., Ordonez, J.A., Tomas-Barberan, F.A., 2003. Phenolic compounds and fatty acids from acorns (*Quercus* spp.): the main dietary constituent of free ranged iberian pigs. J. Agric. Food Chem. 51, 6248–6255.

Corsi, L., Avallone, R., Cosenza, F., Farina, F., Baraldi, C., Baraldi, M., 2002. Antiproliferative effects of *Ceratonia siliqua* L. on mouse hepatocellular carcinoma cell line. Fitoterapia 73, 674–684.

Custódio, L., Patarra, J., Albericio, F., Neng, N.R., Nogueira, J.M.F., Romano, A., 2013. Extracts from *Quercus* sp: acorns exhibit *in vitro* neuroprotective features through inhibition of cholinesterase and protection of the human dopaminergic cell line SH-SY5Y from hydrogen peroxide-induced cytotoxicity. Ind. Crops Prod. 45, 114–120.

Dastmalchi, K., Damien Dorman, H.J., Oinonen, P.P., Darwis, Y., Laakso, I., Hiltunen, R., 2008. Chemical composition and *in vitro* antioxidative activity of a lemonbalm (*Melissa officinalis* L.) extract. LWT 41, 391–400.

Feng, Y.C., Li, W.L., He, F., Kong, T., Huang, X., Gao, Z., Lu, N., Li, H., 2015. Aqueous two-phase system as an effective tool for purification of phenolic compounds from fig fruits (*Ficus carica* L. Sep. Sci. Technol. 50, 1785–1793.

Fernández-Lara, R., Gordillo, B., Rodríguez-Pulido, F.J., González-Miret, M.L., del Villar-Martínez, A.A., Dávila-Ortiz, G., Heredia, F.J., 2015. Assessment of the differences in the phenolic composition and color characteristics of new strawberry (*Fragaria x ananassa* Duch.) cultivars by HPLC-MS and imaging tristimulus colorimetry. Food Res. Int. 76, 645–653.

Franck, T., Mouithys-Mickalad, A., Robert, T., Ghitti, G., Deby-Dupont, G., Neven, P., Serteyn, D., 2013. Differentiation between stoichiometric and anticatalytic antioxidant properties of benzoic acid analogues: a structure/redox potential relationship study. Chem. Biol. Interact. 206, 194–203.

Gordillo, B., Rodríguez-Pulido, F.J., Gonzaílez-Miret, M.L., Quijada-Morín, N., Rivas-Gonzalo, J., García-Esteívez, I., Heredia, F.J., Escribano-Bailoín, M.T., 2015. Application of differential colorimetry to evaluate anthocyanin-flavonol-flavanol ternary copigmentation interactions in model solutions. J. Agric. Food Chem. 63, 7645–7653.

Gülçin, I., Küfrevioğlu, O.I., Oktay, M., Büyükokuroğlu, M.E., 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J. Ethnopharmacol. 90, 205–215.

Gu, H.F., Li, C.M., Xu, Y.J., Hu, W.F., Chen M. h. Wan, Q., 2008. Structural features and antioxidant activity of tannin from persimmon pulp. Food Res. Int. 41, 208–217.

Gubbuk, H., Kafkas, E., Guven, D., Gunes, E., 2010. Physical and phytochemical profile of wild and domesticated carob (*Ceratonia siliqua* L.) genotypes. Span. J. Agric. Res. 8 (4), 1129–1136.

Hasegawa, H., Suzuki, K., Nakaji, S., Sugawara, K., 1997. Analysis and assessment of the capacity of neutrophils to produce reactive oxygen species in a 96-well microplate format using lucigenin and luminal dependent chemiluminescence. J. Immunol. Methods 210, 1–10.

Havlik, J., Huebra, R.G., Hejtmankova, K., Fernandez, J., Simonova, J., Melich, M., Rada, V., 2010. Xanthine oxidase inhibitory properties of Czech medicinal plants. J. Ethnopharmacol. 132, 461–465.
Huang, D.J., Lin, C.D., Chen, H.J., Lin, Y.H., 2004. Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57′) constituents. Bot. Bull. Acad. Sin. 45, 179–186.
Karioti, A., Bilia, A.R., Skaltsa, H., 2010. *Quercus ilex* L.: a rich source of polyacylated flavonoid glucosides. Food Chem. 123, 131–142.
Kim, H.J., Lee, J.Y., Kim, S.M., Park, D.A., Jin, C., Hong, S.P., Lee, Y.S., 2009. A new epicatechin gallate and calpain inhibitory activity from Orostachys japonicas. Fitoterapia 80, 73–76.
Lin, C.M., Chen, C.S., Chen, C.T., Liang, Y.C., Lin, J.K., 2002. Molecular modelling of flavonoids that inhibit xanthine oxidase. Biochem. Biophys. Res. Commun. 294 (1), 167–172.
Lloki Assanga, S.B., Gil-Salido, A.A., Lewis Luján, L.M., Rosas-Durazo, A., Acosta-Silva, A.L., Rivera-Castañeda, E.G., Rubio-Pino, J.L., 2013. Cell growth curves for different cell lines and their relationship with biological activities. Int. J. Biotechnol. Mol. Biol. Res. 4 (4), 60–70.
Maksimović, Z., Malencic, D., Kovacevic, N., 2005. Polyphenol contents and antioxidant activity of Maydis stigma extracts. Bioresour. Technol. 96, 873–877.
Marrelli, M., Menichini, F., Statti, G.A., Bonesi, M., Duez, P., Menichini, F., Conforti, F., 2012. Changes in the phenolic and lipophilic composition: in the enzyme inhibition and antiproliferative activity of *Ficus carica* L. cultivar Dottato fruits during maturation. Food Chem. Toxicol. 50, 726–733.
Mau, J.L., Tsai, S.Y., Tseng, Y.H., Huang, S.J., 2005. Antioxidant properties of methanolic extracts from *Ganoderma tsugae*. Food Chem. 93, 641–649.
Melendez-Martinez, A.J., Vicario, I.M., Heredia, F.J., 2007. Provitamin A carotenoids and ascorbic acid contents of the different types of orange juices marketed in Spain. Food Chem. 101, 177–184.
Mossman, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.
Park, E.J., Choi, K.S., Kwon, T.K., 2011. beta-Lapachone-induced reactive oxygen species (ROS) generation mediates autophagic cell death in glioma U87 MG cells. Chem. Biol. Interact. 189, 37–44.
Pelicano, H., Carney, D., Huang, P., 2004. ROS stress in cancer cells and therapeutic implications. Drug Resist. Updat. 7 (2), 97–110.
Rodriguez-Bernaldo de Quiros, A., Costa, H.S., 2006. Analysis of carotenoids in vegetable and plasma samples: a review. J. Food Comp. Anal. 19, 97–111.
Roseiro, L.B., Tavares, C.S., Roseiro, J.C., Rauter, A.P., 2013a. Antioxidants from aqueous decoction of carob pods biomass (*Ceretonia siliqua* L:): optimisation using response surface methodology and phenolic profile by capillary electrophoresis. Ind. Crops Prod. 44, 119–126.
Roseiro, L.B., Duarte, L.C., Oliveira, D.L., Roque, R., Bernardo-Gil, M.G., Martins, A.I., Sepúlveda, C., Almeida, J., Meireles, M., Gírio, F.M., Rauter, A.P., 2013b. Supercritical: ultrasound and conventional extracts from carob (Ceratonia siliqua L.) biomass: effect on the phenolic profile and antiproliferative activity. Ind. Crops Prod. 47, 132–138.
Rosser, B.G., Powers, S.P., Gores, G.J., 1993. Calpain activity increases in hepatocytes following addition of ATP: demonstration by a novel fluorescent approach. J. Biol. Chem. 268, 23593–23600.
Sęczyk, L., Świeca, M., Gawlik-Dziki, U., 2016. Effect of carob (*Ceratonia siliqua* L.) flour on the antioxidant potential nutritional quality, and sensory characteristics of fortified durum wheat pasta. Food Chem. 194, 637–642.
Simões, L.M.C., Gregório, L.E., Da Silva Filho, A.A., de Souza, M.L., Azzolini, A.E.C.S., Bastos, J.K., Lucisano-Valim, Y.M., 2004. Effect of Brazilian green propolis on the production of reactive oxygen species by stimulated neutrophils. J. Ethnopharmacol. 94, 59–65.
Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics whith phosphomolibdie-phosphotungstic acids reagents. Am. J. Enol. Vitic. 16, 144–158.
Sohretoglu, D., Sabuncuoglu, S., Harput, U.S., 2012. Evaluation of antioxidative, protective effect against H_2O_2 induced cytotoxicity, and cytotoxic activities of three different *Quercus* species. Food Chem. Toxicol. 50, 141–146.Soltana, H., Tekaya, M., Amri, Z., El-Gharbi, S., Nakbi, A., Harzallah, A., Mechri, B., Hammami, M., 2016. Characterization of fig achenes' oil of Ficus carica grown in Tunisia. Food Chem. 196, 1125–1130.
Tazzeo, T., Worek, F., Janssen, L.J., 2009. The NADPH oxidase inhibitor diphenyleneiodonium is also a potent inhibitor of cholinesterases and the internal Ca^{2+} pump. Br. J. Pharmacol. 158, 790–796.
Tovar, M.J., Romero, M.P., Girona, J., Motilva, M.J., 2002. L-Phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L. *cv Arbequina*) fruit grown under different irrigation regimes. J. Sci. Food Agric. 82, 892–898.
Vijaya Kumar Reddy, C., Sreeramulu, D., Raghunath, M., 2010. Antioxidant activity of fresh and dry fruits commonly consumed in India. Food Res. Int. 43, 285–288.
Vitali Čepo, D., Mornar, A., Nigovic, B., Kremer, D., Radanovic, D., Dragojevic, I.V., 2014. Optimization of roasting conditions as an useful approach for increasing antioxidant activity of carob powder. LWT Food Sci. Technol. 58, 578–586.
Yemiş, O., Bakkalbaşi, E., Artık, N., 2012. Changes in pigment profile and surface colour of fig (*Ficus carica* L.) during drying. Int. J. Food Sci. Technol. 47, 1710–1719.