Industrial Crops and Products 53 (2014) 6–15

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities

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ARTICLE INFO

Article history:
Received 28 October 2013
Received in revised form
28 November 2013
Accepted 2 December 2013

Keywords:
Cyclooxygenase-1
Acetylcholinesterase
Medicinal plants
Phenolic compounds
Antioxidant activities

ABSTRACT

Extracts of nine medicinal plants were screened for their anti-inflammatory activity using the cyclooxygenase-1 assay and their acetylcholinesterase inhibitory effect. The antioxidant activity was assessed by four methods: free radicals DPPH• (1,1-diphenyl-2-picrilhydrazyl), nitric oxide assay, β-carotene bleaching test and metal chelating power. The amounts of different phenolic compounds were also determined. *Myrtus communis* (leaves), *Pistacia lentiscus* (leaves) and *Globularia alypum* (flowers) presented the highest amounts of total phenolic compounds while the concentrations of total flavonoids, flavonols, proanthocyanidins and total tannins varied with plant species. *Marrubium vulgare* (leaves) gave the best inhibitory activity of the enzyme Cox-1 with an IC_{50} of 0.082 mg/ml which was statistically not different from the standard indomethacin (0.061 mg/ml). The best anti-acetylcholinesterase activity was exhibited by the leaf extracts of *M. communis*, *P. lentiscus* and *Eryngium maritimum*, 92.38, 73.84 and 65.34%, respectively. In the DPPH assay, *P. lentiscus* and *M. communis* presented the best activity and their inhibitions were not different from each other ($^*p < 0.05$) but were significantly different from the pure standards rutin and BHA. Among the tested plants, *Scilla maritima* presented the best nitric oxide scavenging activity. In the β-carotene assay, extracts of *M. communis* leaves and fruits and *P. lentiscus* leaves were the most potent with 63.60, 47.61 and 43.02%, respectively. Metal chelating activity assay showed that *E. maritimum* leaves and stem and *M. communis* leaves had the best chelating power, 49.78, 32.32 and 35.98%, respectively.

1. Introduction

Non-steroid anti-inflammatory drugs (NSAIDs) are of huge therapeutic benefit in the treatment of inflammatory diseases since they are widely used for the treatment of pain, inflammation and fever (Vonkeman and van de Laar, 2010). The main mechanism of action of these drugs is believed to be the inhibition of the cyclooxygenase enzymes: the constitutive cyclooxygenase-1 (Cox-1) and the inducible cyclooxygenase-2 (Cox-2) (Ulbrich et al., 2002). Cyclooxygenase (Cox) is a bi-functional enzyme that first catalyzes the addition of two molecules of oxygen to arachidonic acid to form the hydroperoxide prostaglandin G_2 (PGG_2), then reduces the hydroperoxide to the alcohol, PGH_2, by a peroxidase activity. Prostaglandins (PGs) are important biological mediators of inflammation, originating from biotransformation of arachidonic acid catalyzed by cyclooxygenase (Gierse et al., 2008). The most common side effects associated with all currently available NSAIDs are gastrointestinal haemorrhagia and ulceration (Dannhardt et al., 2000). These side effects during anti-inflammatory therapy are caused by interference with the physiological properties of prostaglandins (Ulbrich et al., 2002). During inflammatory responses, the activation of phospholipase A_2 induces the mobilization of fatty acids, in particular arachidonic acid from the membrane lipid pool (Fiorucci et al., 2001; Fawole et al., 2010). Arachidonic acid is then oxidized by Cox-1 or Cox-2 enzymes, leading to the production of prostaglandins (Fiorucci et al., 2001). In addition, oxidants such as reactive oxygen species (ROS) generated from activated neutrophils and macrophages have been reported to play an important role in the pathogenesis of various pain-related diseases, including neurodegenerative disorders, like Alzheimer's disease (AD) (Gibson and Huang, 2005; Fawole et al., 2010). This disease is frequent in elderly people, as a result of malfunctioning of different biochemical pathways. The drugs approved for the AD therapy act by counteracting the

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http://dx.doi.org/10.1016/j.indcrop.2013.12.008

Table 1
Botanical and common names, families, voucher specimens, plant parts and medicinal properties of the investigated plants.

Family	Species	Local name	Voucher specimen	Used part	Traditional uses
Myrtaceae	*Myrtus communis*	Chilmoune	D-PH-2013-37-6 D-PH-2013-37-12	Leaves Fruits	Antiseptic, disinfectant drug and hypoglycemic effects, antimicrobial, tonic and balsamic agent, Inhibit xanthine oxidase activity, anti-inflammatory activity
Apiaceae	*Eryngium maritimum*	Tabelyatut	D-PH-2013-37-1 D-PH-2013-37-16	Leaves Stem	Diuretic, antiscorbutic, a cytotonic, a urethritis remedy, a stone inhibitor, an aphrodisiac, an expectorant, an anthelmintic, antinociceptive and anti-inflammatory activity. Used for snakebites, fevers, or female reproductive disorders
Anacardiaceae	*Pistacia lentiscus*	Amadagh	D-PH-2013-37-5	Leaves	Antibacterial, and antiulcer agent, treatment of eczema, diarrhea and throat infections, anti-inflammatory, antipyretic and insecticidal activities
Globulariaceae	*Globularia alypum*	Taselgha	D-PH-2013-37-7 D-PH-2013-37-17	Leaves Flowers	Hypoglycemic, laxative and diuretic agent, treatment of rheumatism, arthritis, hemorrhoids. Anti-inflammatory activity
Lamiaceae	*Marrubium vulgare*	Marruyet	D-PH-2013-37-2	Leaves	Treatment of gastroenterical and respiratory diseases, antinociceptive, anti-inflammatory, hypoglycemic and insecticidal effects. Tonic, aromatic, expectorant, diaphoretic and diuretic activities
Liliaceae	*Scilla maritima*	Lebsel wuchen	D-PH-2013-37-14	Bulb	Treatment of heart insufficiency, edema and bad kidney performance, memory-enhancement and rodenticide. Anti-inflammatory activity, cardiotonic, diuretic

acetylcholine deficit, that is, they try to enhance the acetylcholine level in the brain (Heinrich and Teoh, 2004). Acetylcholine is involved in the signal transfer in the synapses. After being delivered in the synapses, acetylcholine is hydrolyzed giving choline and acetyl group in a reaction catalyzed by the enzyme acetylcholinesterase. The molecular basis of the Alzheimer drugs used so far, take advantage of their action as acetylcholinesterase inhibitors (AChEIs) (Heinrich and Teoh, 2004; Ferreira et al., 2006) but these drugs have been reported to have their adverse effects including gastrointestinal disturbances, hepatotoxicity, nausea, vomiting, diarrhea, dizziness and problems associated with bioavailability (Schulz, 2003; Mukherjee et al., 2007), which increases the interest in finding better acetylcholinesterase inhibitors from natural resources.

Considering the developing increasing demand for plant-derived drugs, the nine selected plants: *Pistacia lentiscus* (leaves), *Myrtus communis* (leaves and fruits), *Globularia alypum* (leaves and flowers), *Marrubium vulgare* (leaves), *Eryngium maritimum* (leaves and stem) and *Scilla maritima* (bulb) could be further assessed and utilized in view of their health benefit effects where some of them are reported in Table 1. Moreover, according to our knowledge, there are no reports on the acetylcholinesterase and cyclooxygenase-1 inhibitory activities of the studied species in Algeria. Therefore, the aims of the present investigation were to screen for antioxidant capacities in the medicinal plant extracts, and to determine their inhibitory effects on the acetylcholinesterase and cyclooxygenase-1 enzymes. To elucidate their oxidative actions, the extracts were subjected to a range of *in vitro* tests, including the metal chelating power, the ability to scavenge DPPH radical, the β-carotene bleaching test and the nitric oxide assay. The amounts of antioxidant components (total phenolic compounds, flavonoids, flavonols, proanthocyanidins and total tannins) from the crude plant extracts were also determined.

2. Material and methods

2.1. Chemicals

All chemicals and reagents were of analytical grade and were supplied from Sigma–Aldrich Química S. A. (Sintra, Portugal) and from Sigma (represented by Algerian Chemical Society, Setif, Algeria).

2.2. Plant materials and samples preparation

Nine medicinal plants were harvested in 2009 from two locations, in remote areas in the suburbs of Taghzouit and Aboudaou, Bajaia City (Algeria). The various data (local name, medicinal uses, used parts of plant, method of preparation and administration) were collected from local inhabitants having knowledge of the curative properties of these plants. Botanical identification was made by the member of laboratory of Botany (Faculty of Life and Nature Sciences, University Abderrahmane Mira of Bejaia) according to "Nouvelle flore de l'Algérie et des regions desertiques et Meridionales" (Quezel and Santa, 1963). Voucher specimens (Table 1) were deposited at the Herbarium of Natural History Museum of Aix-en-Provence, France. Fresh leaves, flowers and stems were air-dried in shade at room temperature and the bulbs of *S. maritima* were peeled, ground and then frozen and lyophilized immediately. After drying and lyophilization, plant material was ground to a fine powder (diameter < 250 μm) using an electric mill (IKA^R A11 basic, Germany) and 1 g of this powder was exhaustively extracted by maceration with 10 ml of methanol, at room temperature for 24 h. In all cases, the solutions were filtered and concentrated to dryness under reduced pressure in a rotary evaporator (40 °C). Dry extracts were stored at −20 °C until used.

2.3. Determination of the amounts of phenolic compounds

2.3.1. Total phenolics content

Total phenolic compounds content was assayed using the Folin–Ciocalteu reagent, following Singleton and Rossi method (1965). An aliquot (1 ml) of diluted sample extract (0.3 mg/ml) was added to 500 μl of the Folin–Ciocalteu reagent and 6 ml of water. The mixture was shaken and allowed to stand for 5 min, before addition of 1.5 ml of Na_2CO_3 (20%). An aliquot of 1.9 ml of distilled water was added and mixed thoroughly. After incubation in dark for 2 h, the absorbance at 760 nm was read *versus* the prepared blank. Total phenolic content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid ($y = 0.9397x$; $R^2 = 0.998$).

2.3.2. Total flavonoids content

Determination of the flavonoids content was achieved using the method described by Huang et al. (2004) by addition of

aluminum chloride reagent to the solution containing the extract. The absorbance was read at 430 nm and concentrations of flavonoids were deduced from a standard curve ($y = 41.686x$; $R^2 = 0.997$) and calculated in mg quercetin equivalent (QE)/g dry weight (DW).

2.3.3. Flavonols content

Total flavonols in the plant extracts were estimated using the method reported by Adedapo et al. (2008). To 2 ml of sample, 2 ml of $AlCl_3$ (2%) ethanol and 3 ml (50 g/L) sodium acetate solutions were added. The mixture was shaken and incubated for 2.5 h at 20 °C. The absorbance was read at 440 nm. Total flavonols content was expressed as mg of quercetin equivalents per g of dry weight (mg EQ/g DW) through the calibration curve with quercetin ($y = 15.16x + 0.0092$; $R^2 = 0.999$).

2.3.4. Proanthocyanidins content

The concentration of proanthocyanidins was determined by butanol–HCl assay (Maksimovic et al., 2005). Extract of plants (0.5 ml) was mixed with 3 ml of butanol–HCl (95:5; v/v) and 0.1 ml of iron sulphate (2 g/100 ml). The mixture was incubated at 90 °C for 1 h. The absorbance was determined at 530 nm. The amount of proanthocyanidins was expressed as mg (+)-catechin equivalent (CE) g^{-1} DW ($y = 2.078x - 0.1488$; $R^2 = 0.999$).

2.3.5. Total tannins content

Total tannins were estimated according to the protocol developed by Hagerman and Butler (1978) on the basis of their precipitation by a protein, bovine serum albumin (BSA). The method is based upon the obtention of a colored complex Fe^{++}–phenols which can be measured spectrophotometrically at 510 nm. The results were expressed as mg equivalent of tannic acid (ETA)/g DW ($y = 0.5184x$; $R^2 = 0.999$).

2.4. Cyclooxygenase-1 assay

The Cox-1 (EC 1.14.99.1) activity was measured by following spectrophotometrically at 611 nm the reaction of oxidation of TMPD with arachidonic acid. This assay was performed according to Copeland et al. (1994), with the following modifications. Briefly, 50 μl of Cox-1 enzyme (200 units/cuvette) and 100 μl of hematin (3 μM in Tris-buffer, pH 8) as co-factor and 1000 μl Tris–HCl buffer (100 mM, pH 8) and 50 μl of DMSO per sample were pre-incubated. This enzyme/co-factor solution was added to the test solution consisting of 50 μl plant extract or an inhibitor, dissolved in DMSO and pre-incubated for 3 min at 25 °C. Then 200 μl of TMPD was added. The reaction was initiated by addition of 50 μl of arachidonic acid to the enzyme/extract mixture and contents were mixed immediately. The initial velocity of the reaction was measured following the reaction of oxidation of TMPD at 611 nm for 20 s. The velocities observed at different inhibitor concentration were divided by the velocity observed for enzyme samples pre-incubated for the same time with 10% (v/v) inhibitor free DMSO, and this ratio was multiplied by 100 to yield percent control activity. IC_{50} values were determined by regression analysis.

$$I(\%) = 100 - \left(\frac{V_{\text{with inhibitor}}}{V_{\text{without inhibitor}}}\right) \times 100$$

2.5. Acetylcholinesterase inhibition

Acetylcholinesterase enzymatic activity was measured by the Ellman test (Ferreira et al., 2006). 400 μl of 50 mM Tris buffer (pH 8), 50 μl of plant extract in methanol with different concentrations and 25 μl of an enzyme solution containing 0.26 U/ml were incubated during 15 min. Subsequently, 75 μl of a solution of acetylthiocholine iodide (AChI) 15 mM and 475 μl of 3 mM 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) were added. Absorbance of the mixture was measured at 405 nm when the reaction reached the equilibrium. A control mixture was prepared, using methanol instead of extract and was considered 100% activity. Inhibition, in %, was calculated in the following way:

$$I(\%) = 100 - \left(\frac{V_{\text{sample}}}{V_{\text{control}}}\right) \times 100$$

where I is the percent inhibition of acetylcholinesterase, V_{sample} is the initial velocity of the extract containing reaction and V_{control} is the initial velocity of the control reaction. A blank with Tris buffer instead of enzyme solution was used. Extract concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against extract solution concentrations.

2.6. Antioxidant capacity

2.6.1. DPPH• radical scavenging activity

The DPPH (1,1-diphenyl-2-picrilhydrazyl) radical scavenging activity of the plant extracts was determined using the method proposed by Katalinic et al. (2006). Aliquot (50 μL) of the tested sample was placed in a cuvette and 2 ml of 6×10^{-5} M methanolic solution of DPPH radical (freshly prepared) was added. The mixture was maintained at room temperature for 16 min then the absorbance was measured at 517 nm against the corresponding blank. Methanolic solutions of pure compounds BHA and rutin were tested too.

The percentage inhibition of the DPPH radical by the samples was calculated by the following formula:

$$I(\%) = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100$$

where I (%) is the percentage inhibition of the DPPH radical, A_{blank} 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. Extract concentration providing 50% inhibition (IC_{50}) was obtained plotting inhibition percentage *versus* extract solution concentrations.

2.6.2. Nitric oxide radical scavenging activity

The ability of the extracts to scavenge nitric oxide free radicals was determined using the method reported by Dastmalchi et al. (2008). In brief, a 0.5 ml aliquot of extract or positive control (quercetin and catechin) dissolved in KH_2PO_4–KOH (50 mmol/l, pH 7.4) was mixed with 0.5 ml of (10 mmol/l) sodium nitroprusside solution. The mixture was incubated at 37 °C for 2.5 h under normal light condition. After incubation, the sample was placed in dark for 20 min. Thereafter, 1 ml of Griess reagent (1 g/l N-(1-naphtyl)ethylenediamine and 10 g/l sulphanilamide dissolved in 20 ml/l aqueous H_3PO_4) was added and the absorbance was taken after 40 min at 546 nm. The percentage inhibition was calculated using the following equation:

$$I(\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

where I (%) is the percentage inhibition of NO 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.

2.6.3. β-Carotene–linoleic acid bleaching inhibition

The ability of the extract to inhibit the bleaching of the β-carotene–linoleic acid emulsion was determined using the method

described by Khadri et al. (2010) with a slight modification. In brief, 2 mg β-carotene dissolved in 1 ml chloroform, 25 μl of linoleic acid and 200 mg of Tween 40 were added and transferred into a round-bottom flask. Once the chloroform had been removed under nitrogen, 50 ml distilled water saturated with oxygen was added the flask with vigorous stirring and the resulting mixture was sonicated for 30 min for homogenization. Thereafter, 2.5 ml aliquots of this emulsion were transferred to a series of tubes containing 300 μl of extract dissolved in ethanol and were further shaken. Absorbance of each sample was recorded at 470 nm immediately after sample preparation (t = 0 min). After that the samples were placed in a water bath at 50 °C for a period of 2 h, together with two blanks, one containing antioxidants (BHT or gallic acid) as a positive control and the other with the same volume of ethanol instead of the extracts. The absorbance of each sample after heating (t = 120 min) was measured.

The antioxidant activity (AA) of the extracts under investigation was expressed as:

$$\mathrm{AA}\,(\%) = \left[1 - \left(\frac{A_{(t=0)} - A_{(t=120)}}{A_{0(t=0)} - A_{0(t=120)}}\right)\right] \times 100$$

where AA is the antioxidant activity, $A_{(t=0)}$ is the absorbance (470 nm) of the solution in investigation at 0 min, $A_{(t=120)}$ is the absorbance (470 nm) of the same solution at t = 120 min, $A_{0(t=0)}$ is the absorbance (470 nm) of the positive control (ethanol without extract) sample at 0 min and $A_{0(t=120)}$ is the absorbance (470 nm) of a the positive control (ethanol without extract) sample at t = 120 min. Extract concentration providing 50% inhibition (IC_{50}) was obtained plotting inhibition percentage *versus* extract solution concentrations.

2.6.4. Metal chelating activity

The ability of the extract to chelate iron (II) was assessed by the method of Carter (1971). In brief, to a 200 μl of dissolved extract was added 100 μl (2 mmol/l) $FeCl_2{\cdot}4H_2O$ and 900 μl methanol. After 5 min incubation, the reaction was initiated by the addition of 400 μl (5 mmol/l) ferrozine. After a further 10 min incubation period, the absorbance of the reaction mixture was measured at 562 nm. The ratio of inhibition of ferrozine–Fe^{2+} complex formation was calculated as follows:

$$\%\,\text{inhibition} = \left[\frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}}\right] \times 100$$

where A_{sample} was the absorbance in the presence of the sample of extracts or the standard EDTA. The concentration at which the extract exerts 50% of its effect (IC_{50}) values was estimated by a linear regression algorithm.

2.7. Statistical analysis

Data were presented as mean ± standard deviation (SD) of three determinations. Statistical analyses were performed using a one-way analysis of variance (ANOVA) in the software STATISTICA 5.5. Differences were considered significant at $^*p < 0.05$.

3. Results and discussion

3.1. Determination of phenolic compounds

Phenolic compounds constitute one of the major groups of compounds known to inhibit some molecular targets of pro-inflammatory mediators in inflammatory responses and to act as primary antioxidants or free radical terminators (Fawole et al., 2009). For these reasons, we have tried to determine their total amounts.

Among analyzed plant extracts, extremely high total phenolic content was detected in *M. communis* (leaves), *P. lentiscus* (leaves) and *G. alypum* (flowers) (Table 2). The lowest total phenolic level was detected in *E. maritimum* (stem). The leaves of *M. communis* contained more phenolic compounds than the fruits and the amounts of flavonols and proanthocyanidins in these two parts were not far from each other. Several works found that *M. communis*, *P. lentiscus* and *G. alypum* were rich in phenolic compounds (Chryssavgi et al., 2008; Aidi Wannes et al., 2010; Djeridane et al., 2010) and our results are in agreement with them.

The leaves of *E. maritimum* and *M. vulgare* presented almost the same amounts of phenolics ($^*p < 0.05$). The colorimetric dosage of flavonols revealed that the extracts contained small amounts of this group. On what concerns proanthocyanidins content, *P. lentiscus* (leaves), *S. maritima* (bulb) and *M. communis* fruits and leaves gave the best results. The amounts of flavonols and proanthocyanidins in *G. alypum* (leaves and flowers) did not present any significant differences between both plant parts; and the same remark for proanthocyanidins content in the leaves of *E. maritimum* and *M. vulgare* can be done. Table 2 showed also that the amounts of total flavonoids varied widely in the investigated plant extracts and ranged from 1.34 to 24.86 mg EQ/g dry extract. The best amounts were obtained by *M. communis* and *E. maritimum* leaves, followed by *P. lentiscus* and *G. alypum* leaves. Recently, with the use of reversed-phase high-performance liquid chromatography hyphenated to mass spectrometry electrospray ionization coupled to quadrupole time-of-flight (RP-HPLC-ESI-QTOF-MS), we found that *P. lentiscus* leaves were rich in flavonoids and phenolic acids (Rodríguez-Pérez et al., 2013).

Another important group investigated in this study was the total content of tannins (Table 2). *P. lentiscus* and *M. communis* gave the best levels of total tannins. Our results are in agreement with those found by Romani et al. (2002) and Cakir (2004) who determined that these plants were rich in tannins.

E. maritimum and *M. vulgare* leaves did not present any significant differences in the total tannins content and these later compounds represented approximately the half content of total tannins. *G. alypum* flowers contained more amounts of tannins than flavonoids and its leaves presented the inverse case. It is important to note that for all the investigated plant extracts, proanthocyanidins contents were very low compared to the total tannins content, this result may suggest that the plants contained more hydrolysable tannins than the condensed ones.

Variation in the amounts of phenolic compounds could be attributed to several reasons. The solubility of phenolic compounds is actually governed by the type of solvent used, the degree of polymerization of phenolics, as well as by the interaction of phenolics with other food constituents and formation of insoluble complex. For that purpose, methanol was recommended and frequently used for the extraction of phenolics (Galvez et al., 2005). Indeed, the phenolic contents of a plant depend on a number of intrinsic (genetic) and extrinsic (environmental, handling and storage) factors (Rapisarda et al., 1999; Fratianni et al., 2007).

3.2. Cyclooxygenase-1 assay

From nine plant extracts, only four have presented anti-inflammatory activity (Fig. 1). Percentages of inhibition obtained at a concentration of 0.033 mg/ml from these plants, in decreased order, were *M. vulgare* leaves (68.15%), *G. alypum* flowers (61.05%), *E. maritimum* leaves (8.16%) and *G. alypum* leaves (5.33%), respectively. No inhibition in this test was found with the extract of *M. communis* (leaves and fruits), *S. maritima* (bulbs), *P. lentiscus* leaves and *E. maritimum* (stem) at this concentration.

M. vulgare leaves and *G. alypum* flowers have given the best and higher inhibitory activity of the enzyme. This activity could

Table 2
Total phenolics, flavonoids, flavonols, proanthocyanidins and tannins content in the medicinal plant extracts.

	TPC (mg GAE/gDW)	Flavonoids (mg QE/gDW)	Flavonols (mg QE/gDW)	Proanthocyanidins (mg CE/gDW)	Total tannins (mg TAE/gDW)
Myrtus communis (leaves)	285.73 ± 2.28^{a}	24.868 ± 0.415^{a}	7.38 ± 0.04^{b,c}	19.10 ± 0.12^{d}	117.670 ± 1.543^{a}
Pistacia lentiscus (leaves)	238.33 ± 3.28^{b}	19.578 ± 0.326^{b}	7.29 ± 0.24^{c}	39.29 ± 0.61^{a}	100.694 ± 2.409^{b}
Globularia alypum (flowers)	156.97 ± 1.14^{c}	1.346 ± 0.023^{h}	3.12 ± 0.41^{e}	14.07 ± 0.83^{e}	7.459 ± 0.223^{f}
Globularia alypum (leaves)	102.29 ± 0.50^{e}	16.592 ± 0.799^{c}	3.26 ± 0.13^{e}	14.18 ± 0.14^{e}	9.388 ± 2.357^{f}
Scilla maritima (bulb)	127.90 ± 1.49^{d}	2.159 ± 0.069^{g}	2.08 ± 0.06^{f}	27.41 ± 0.23^{b}	11.703 ± 0.589^{e}
Myrtus communis (fruits)	91.34 ± 3.92^{f}	7.170 ± 0.083^{e}	4.24 ± 0.14^{d}	22.13 ± 0.34^{c}	28.807 ± 0.803^{c,d}
Eryngium maritimum (leaves)	43.83 ± 1.,32^{g}	24.309 ± 0.262^{a}	7.63 ± 0.22^{b}	11.97 ± 0.14^{f}	33.179 ± 1.391^{c}
Marrubium vulgare (leaves)	40.57 ± 1.91^{g}	10.249 ± 0.083^{d}	10.48 ± 0.19^{a}	12.28 ± 0.13^{f}	32.407 ± 0.386^{c}
Eryngium maritimum (stem)	16.49 ± 0.19^{h}	3.505 ± 0.061^{f}	1.04 ± 0.02^{g}	10.12 ± 0.10^{g}	17.747 ± 21.050^{d,e}

Averages ± standard deviation was obtained from three different experiments. GAE, gallic acid equivalents; QE, quercetin equivalents; CE, catechin equivalents; TAE, tannic acid equivalents; DW, dry weight. Means followed by the same letter are not different according to ANOVA (analysis of variance) ($^{*}p < 0.05$). The results are sorted in decreasing order: a > b > c > d > e > f > g > h.

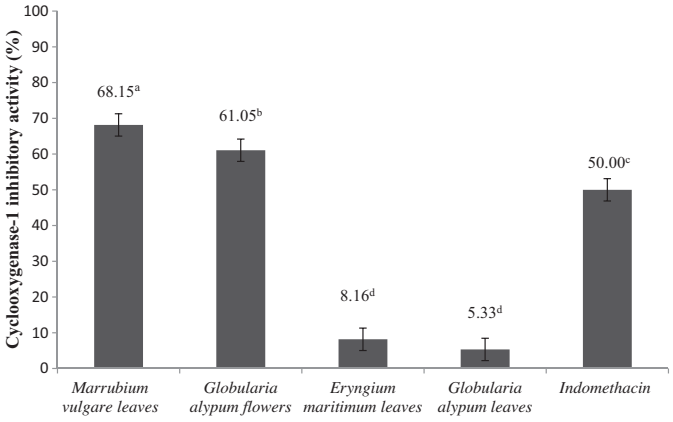

Fig. 1. Cyclooxygenase inhibitory activity of the different plant extracts (concentration of plant extracts is 33 μg/ml). Data were analyzed using one-way ANOVA (analysis of variance). Different letter(s) indicate the values are significantly different ($^{*}p < 0.05$).

be attributed to their polyphenolic compounds (*i.e.* tannins and proanthocyanidins). These compounds are known to inhibit some molecular targets of pro-inflammatory mediators in inflammatory responses (Fawole et al., 2010). Whereas, the results obtained for *E. maritimum* and *G. alypum* leaves were too lower compared to the two preceding plants and they were statistically not different from each other at ($^{*}p < 0.05$). In addition, the anti-inflammatory activity obtained from the flowers of *G. alypum* was much higher compared to its leaves, which leads to say that the inhibitor agents were present in the flowers part.

From the obtained results, the IC_{50} values were determined for *M. vulgare* leaves and *G. alypum* flowers as well as the standard indomethacin. The anti-inflammatory activity of the samples was found to increase dose-dependently and the results were depicted in Table 3.

M. vulgare leaves extract has presented a potent activity with an IC_{50} of 0.082 mg/ml which was statistically not different from the chemical standard indomethacin (0.061 mg/ml).

In the literature, several reports have described that *M. vulgare* and *G. alypum* contained some active components such as phenolic compounds, flavonoids, coumarins, phenylethanoid glycosides, iridoids and monoterpenes. Some authors (Sahpaz et al., 2002; Berrougui et al., 2006; Taskova et al., 2006; Dussossoy et al., 2011) affirm that this variety of molecules could be responsible for the cyclooxygenase inhibition.

Since the extracts of medicinal plants under study were rich in polyphenols, flavonoids and tannins, the data from Table 2 reporting the evaluation of these two class of compounds supported the inhibition of Cox-1.

3.3. Acetylcholinesterase inhibitory activity

Medicinal plant extracts were tested to determine their ability as acetylcholinesterase inhibitors and results were depicted in Fig. 2. For each plant, the inhibition capacity showed the following order: *M. communis* leaves > *P. lentiscus* leaves > *E. maritimum* leaves > *G. alypum* flowers > *M. vulgare* leaves > *S. maritima* bulb > *M. communis* fruits > *G. alypum* leaves.

At a concentration of 0.5 mg/ml for each extract, the percentage inhibition of acetylcholinesterase ranged from 26 to 92%. The best inhibitory activity was exhibited by the leaf extract of *M. communis* followed by the leaves of *P. lentiscus* and *E. maritimum* which were not significantly different ($^{*}p < 0.05$). Extracts of *M. vulgare* leaves, *G. alypum* flowers and *S. maritima* bulb have presented about 50% of inhibition of the enzyme at the tested concentration. As *G. alypum* leaves, the extract of *M. communis* fruits presented the same capacity of inhibition, but in fact, this two plants showed lower values than all the other extracts. In addition, the fruits of *M. communis* were less potent than its leaves. Concerning *E. maritimum* stem, it was not possible to test its extract due to appearance of turbidity in the test conditions.

Our results were within the range of the values found in the literature for the inhibition of this enzyme with plant extracts (Orhan et al., 2009) and were higher than those recently reported for other Lamiaceae and Fumariaceae species (Mukherjee et al., 2007; Loïzzo et al., 2010; Adewusi et al., 2011). The results obtained for the three first plant extracts (cited in order) were better than those found for the standard galantamin (48.80% at 1 mg/ml) used in the work of Orhan et al. (2004).

In this study, AChE inhibitor activity of extracts was found to increase dose-dependently, the results expressed as IC_{50} values were calculated from the regression equations obtained from the activity of samples at different concentrations. The results were presented in Table 3.

The IC_{50} values found for *M. communis* and *P. lentiscus* leaves were higher than those found for the standard compounds (chlorogenic acid, rutin, hyperoside, isoquercitrin and quercitrin) used by Hernandez et al. (2010).

The obtained results showed that eight methanolic plant extracts contained some level of inhibitory activity against AChE, this may suggest that organic solvents were able to extract more active compounds with possible AChE inhibitory activity. These results were in concordance with those found by Orhan et al. (2004) who used acetone extracts and obtained values of inhibition (13.25 to 96.89% at 1 mg/ml concentration of the extract), as well as Adewusi et al. (2011) who used DCM:MeOH extracts and obtained values of inhibition represented by IC_{50} (from 0.03 mg/l to 1 mg/ml).

The best inhibitory activity obtained with *M. communis*, *P. lentiscus* and *E. maritimum* leaf extracts could be attributed to

Table 3
Cyclooxygenase-1 assay, acetylcholinesterase activity, radical scavenging activity, β-carotene bleaching assay and metal chelating power represented by IC_{50} (mg/ml).

	Cox-1 IC_{50}	DPPH IC_{50}	AChE activity	β-Carotene inhibition	Metal chelating activity
Myrtus communis (leaves)		0.003 ± 0.000^{d}	0.03 ± 0.00^{f}	0.07 ± 0.00^{h}	1.24 ± 0.03^{b}
Pistacia lentiscus (leaves)		0.008 ± 0.000^{d}	0.01 ± 0.00^{f}	0.12 ± 0.00^{g}	ND
Globularia alypum (flowers)	0.122 ± 0.006^{a}	0.004 ± 0.001^{d}	0.53 ± 0.05^{c}	0.26 ± 0.02^{e}	ND
Globularia alypum (leaves)		0.031 ± 0.004^{c}	0.82 ± 0.05^{a}	0.51 ± 0.03^{b}	ND
Scilla maritima (bulb)		0.028 ± 0.003^{c}	0.62 ± 0.03^{b}	0.17 ± 0.01^{f}	ND
Myrtus communis (fruits)		0.005 ± 0.001^{d}	0.81 ± 0.02^{a}	0.12 ± 0.01^{g}	ND
Eryngium maritimum (leaves)		0.043 ± 0.005^{b}	0.42 ± 0.03^{d}	0.31 ± 0.02^{d}	1.17 ± 0.05^{c}
Marrubium vulgare (leaves)	0.082 ± 0.004^{b}	0.083 ± 0.004^{a}	0.52 ± 0.03^{c}	0.25 ± 0.00^{e}	1.41 ± 0.01^{a}
Eryngium maritimum (stem)		0.087 ± 0.006^{a}	ND	0.43 ± 0.03^{c}	0.43 ± 0.00^{d}
Indomethacin	0.061 ± 0.009^{c}				
Gallic acid			ND	0.83 ± 0.04^{a}	ND
BHT				0.01 ± 0.00^{i}	ND
EDTA					0.016 ± 0.00^{e}
Galantamine			0.21 ± 0.02^{e}		

Averages ± standard deviation was obtained from three different experiments. Means followed by the same letter are not different according to ANOVA (analysis of variance) ($p < 0.05$). ND, not determined. No correlation between activity and concentration was obtained. IC_{50} of galantamine is expressed in μg/ml.

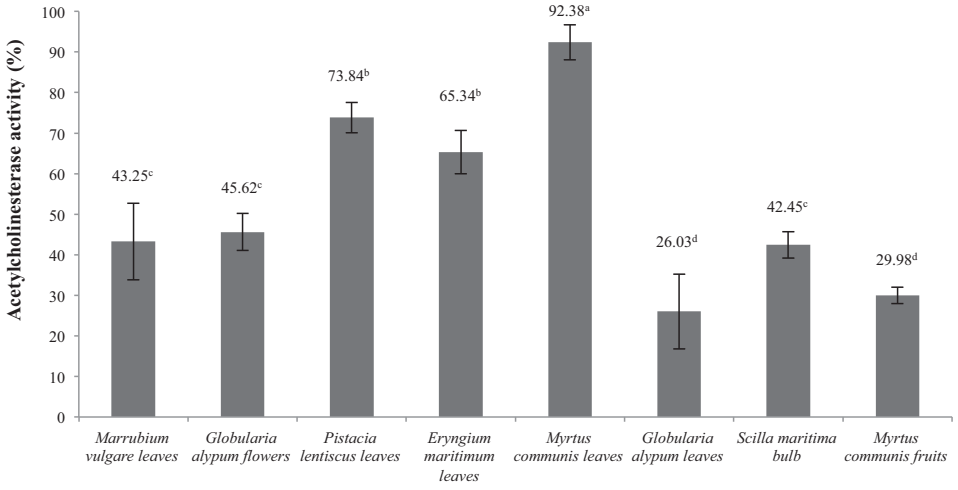

Fig. 2. Acetylcholinesterase inhibitory activity of the different organ plant extracts. Data were analyzed using one-way ANOVA (analysis of variance). Different letter(s) indicate the values are significantly different ($^{*}p < 0.05$).

their chemical composition which mainly contain flavonoids, phenolic acids and tannins, as well as, the possible synergistic interaction between these components. Several studies have shown that flavonoids and other phenolic compounds possess anti-acetylcholinesterase activity (Hostettmann et al., 2006; Fawole et al., 2010; Hernandez et al., 2010). So, in our study, correlation coefficients were found between acetylcholinesterase activity and total flavonols, proanthocyanidins and phenolic contents, 0.66, 0.53 and 0.53, respectively.

3.4. Determination of antioxidant capacities

3.4.1. DPPH• radical scavenging activity

In this study, antioxidant capacity was determined by the DPPH• radical scavenging activity. This method was chosen due to the fact that DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelating and enzyme inhibition as reported by Amarowicz et al. (2004). As shown in Fig. 3, all the tested plants presented more than 50% of radical inhibition, except *E. maritimum* which showed 48.94%. These results revealed that considerable antiradical components were extracted from the different plant parts. Differences in polarity and thus different extractability of the antioxidative components may also explain the differences in antioxidant activity of the extracts. *P. lentiscus* and *M. communis* have presented the best activity and their inhibition were not different from each other ($^{*}p < 0.05$) but were significantly different from the pure standards rutin and the synthetic compound BHA which is used as a food preservative. The ability of the leaf extracts of *P. lentiscus* and *M. communis* to scavenge free radicals could be attributed to their high phenolics contents. Amarowicz et al. (2010) showed a marked antiradical activity of tannins. Results from Table 2 revealed that these plants contained high levels of tannins and proanthocyanidins. Several works have reported the same results (Romani et al., 2002; Chryssavgi et al., 2008; Aidi Wannes et al., 2010).

The leaves of *M. communis* and *E. maritimum* presented higher antioxidant activity than their fruits and stem, respectively. While the flowers of *G. alypum* were more potent than its leaves with an $IC_{50} = 4$ μg/ml (see Table 3). The scavenging potency of these later was higher than found by Djeridane et al. (2010) with an $IC_{50} = 8.7$ mg/ml. The obtained results (Table 2) also showed that this plant contained a good amount of flavonoids and tannins. A lot of work confirmed our results (Djeridane et al., 2006; Es-Safi et al., 2006). The correlation coefficient between phenolics (tannins and proanthocyanidins) and the % of the DPPH• scavenging activity was significant ($r = 0.63$ for both), indicating that polyphenolics may play an important role in free radical scavenging.

3.4.2. Nitric oxide radical scavenging activity

Crude extracts of the studied plants were checked for their inhibitory effect on nitric oxide production. The obtained results were depicted in Fig. 4.

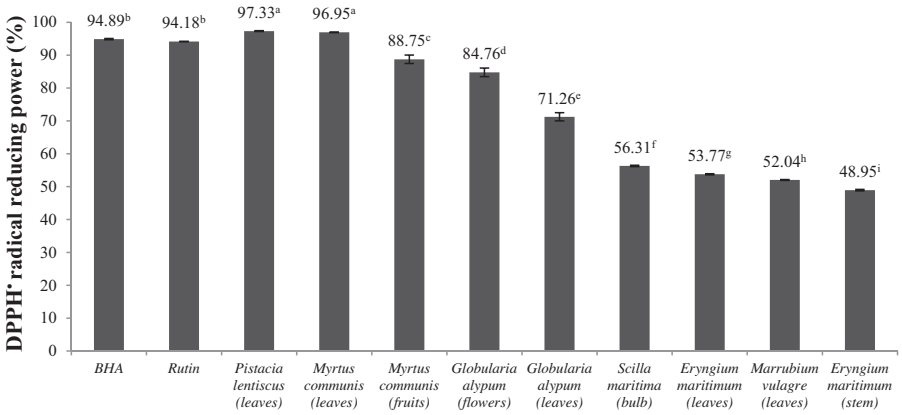

Fig. 3. DPPH radical scavenging activity of the plant extracts with 100 μg/ml. Data were analyzed using one-way ANOVA (analysis of variance). Different letter(s) indicate the values are significantly different ($^*p < 0.05$).

Among the tested plants, *S. maritima* presented the best scavenging activity followed by *E. maritimum* (leaves), *M. communis* (fruits) and *G. alypum* (flowers) with 32.06, 22.07, 15.54 and 14.41%, respectively. Other plants have presented less than 10% of the activity. *S. maritima* bulb have presented the half activity of the pure standard catechin and quercetin. According to our results, this plant was found to contain total tannins and proanthocyanidins more than flavonoids, and consequently, these compounds may contribute to its marked activity. *M. communis* (fruits) and *G. alypum* (flowers) have not presented any significant differences between them ($^*p < 0.05$). They were found to have better activity than their leaves which contained more phenolic compounds. Despite the high content of phenolic compounds, *P. lentiscus* and *M. communis* leaves were less active. Variations in the NO• radical-scavenging activity between these plants were directly related to the structural characteristics and the amount of phenolic constituents present in plant materials. Also, interaction between various phytochemicals contributed to the overall scavenging activity of plant extracts.

3.4.3. Antioxidant activity in the β-carotene–linoleate model system

The potential of the extracts to prevent or minimize the rapid discoloration of the β-carotene was presented in Fig. 5. As can be seen, the antioxidant activity was observed in all screened plant extracts. Antioxidant activity increased with increasing of the extract concentrations. At a concentration of 0.10 mg/ml the activity ranged from 3.35 to 63.60%. *M. communis* (leaves and fruits) and *P. lentiscus* (leaves) extracts demonstrated the best ability to inhibit the bleaching of β-carotene by scavenging linoleate-derived free radicals. It is important to note that the antioxidant activity of the extracts of *M. communis* fruits and *P. lentiscus* leaves were statistically indistinguishable ($^*p < 0.05$) from each other. The same observation was found for the extracts of *G. alypum* flowers, *M. vulgare* and *E. maritimum* leaves at ($^*p < 0.05$), but the inhibitory activity was very low compared with the first plants. These similar activities may imply the presence of approximately similar amounts of lipophilic antioxidants in all three species. Different organs from the same plant were tested, as was the case of *G. alypum*, *M. communis* and *E. maritimum*. The results obtained indicated that the extracts obtained from the leaves presented higher inhibition than other organs which leads to say that lipophilic antioxidant were more important in this part. According to our literature search, there are limited numbers of reports on *S. maritima* (bulb) and *E. maritimum* (leaves and stem). Since the activities of these two species evaluated here are not available in the literature, data presented here could be assumed as the first report.

In this work, we found that plant extracts having potential to prevent or minimize the rapid discoloration of the β-carotene contain high phenolic amounts and coefficient correlation between these compounds and the activity was very high ($r = 0.75$). Some authors have also found a correlation between them (Velioglu et al., 1998; Ozsoy et al., 2008). The presence of a phenolic antioxidant can hinder the extent of β-carotene destruction by "neutralizing" the linoleate free radical (*i.e.* utilizing its redox potential) and any other free radicals formed within the system. Further, interactions may occur between the extract components. The synergism of

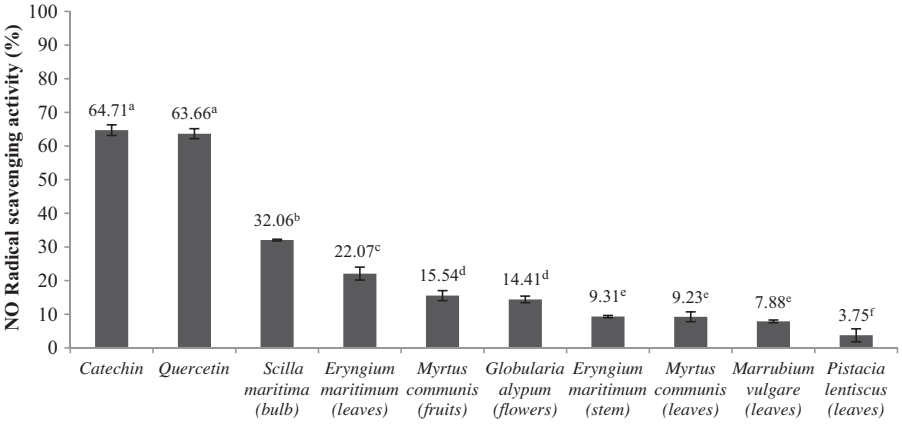

Fig. 4. Nitric oxide scavenging activity of the plant extracts with 0.5 mg/ml. Data were analyzed using one-way ANOVA (analysis of variance). Different letter(s) indicate the values are significantly different ($^*p < 0.05$).

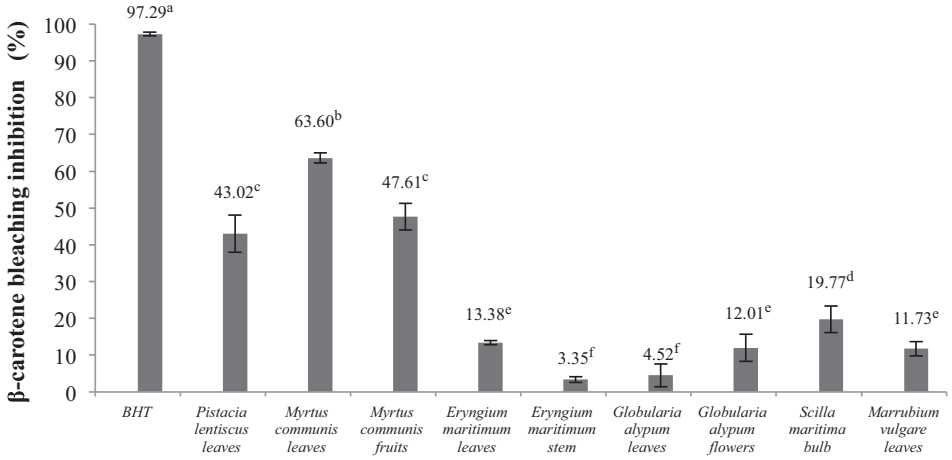

Fig. 5. β-carotene bleaching inhibition of the methanolic plant extracts. Data were analyzed using one-way ANOVA (analysis of variance). Different letter(s) indicate the values are significantly different ($^*p < 0.05$).

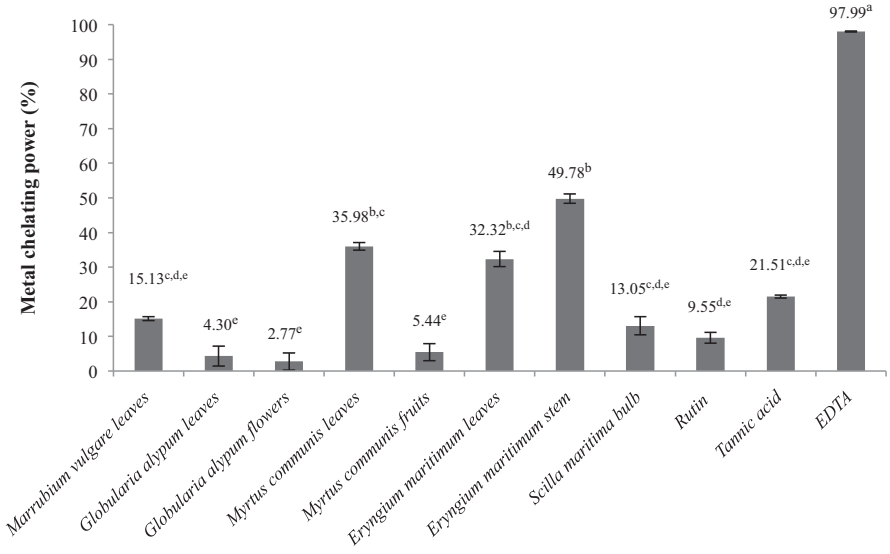

Fig. 6. Metal chelating power of the different medicinal plant extracts. Data were analyzed using one-way ANOVA (analysis of variance). Different letter(s) indicate the values are significantly different ($^*p < 0.05$).

polyphenolics, with one another and/or other components present in an extract may contribute to the overall observed antioxidant activity.

3.4.4. Metal chelating activity

The formation of Fe^{2+}–ferrozine complex is not completed in the presence of plant extracts and standards. At 0.5 mg ml^{-1} concentration, the percentage of metal chelating capacity of the plants ranged from 2.77 to 49.78% (Fig. 6).

Most of the plant extracts interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and can capture ferrous ion forming a more stable complex than ferrozine. The highest ferrous ion-chelating power was observed in extract of *E. maritimum* (stem and leaves), followed by the leaves of *M. communis*. These results could be attributed to their total phenolics and proanthocyanidins content suggesting their ability as a peroxidation protector that relates to the iron binding capacity. Shyu et al. (2009) have found high correlation between the contents of phenolic compounds and ferrous ion chelating power ($r^2 > 0.83$).

The absorbance decreased linearly in the presence of *M. vulgare* and *S. maritima* extracts as well as the standards (rutin and tannic acid), which indicated that the formation of Fe^{2+}–ferrozine complex was not completed. No significant difference ($^*p < 0.05$) between them for iron chelation. The chelating activity of the leaves of *M. communis* and *E. maritimum* as well as its stem was significantly higher ($^*p < 0.05$) than that of the of the standard tannic acid and rutin and demonstrate a marked capacity for iron binding.

Except *E. maritimum*, in all species were leaves and another part of the plant was investigated, the leaves showed always the highest antioxidant activity in this test indicating that is the organ of the plant were antioxidants accumulated. Among all the extracts screened in this study, *G. alypum* (leaves and flowers) and *M. communis* fruits displayed the lowest ferrous ion-chelating power despite their phenolic contents and the extract of *P. lentiscus* was totally inactive.

4. Conclusion

From the present work, we can conclude that among plant extracts, the best amounts of total phenolics, total tannins and

proanthocyanidins were detected in *M. communis* (leaves), *P. lentiscus* (leaves) and *G. alypum* (flowers). The amounts of total flavonoids varied widely in the investigated plant extracts and were higher in *M. communis* and *E. maritimum* leaves. These findings may confirm the interesting potential of these plants as a valuable source of natural bioactive molecules. Percentages of cyclooxygenase-1 inhibition showed that *Marrubium vulgare* leaves extract had the best and the potent activity while the best anti-acetylcholinesterase activity was exhibited by the extract of *M. communis*, *P. lentiscus* and *E. maritimum* leaves. In the β-carotene bleaching test, *M. communis* (leaves and fruits) and *P. lentiscus* (leaves) extracts demonstrated the best inhibition activity while the highest ferrous ion-chelating power was found with *E. maritimum* (stem and leaves) and *M. communis* leaves. The high antioxidant activity exhibited by the selected plants in these assays suggests that they have a potential for use in foods particularly those containing emulsified oils. Almost all the tested plants presented more than 50% of radical inhibition and *P. lentiscus* and *M. communis* have presented the best activity. The $NO^{\bullet}$ scavenging activity showed that *S. maritima* presented the best scavenging activity and presented the half activity of the pure standard catechin and quercetin. On the whole, it is interesting to note that the studied plants have, in fact, properties that may suggest applications in pharmaceutical industry and food. Therefore, the obtained results are useful to further research such as the identification of specific phenolic compounds responsible for the acetylcholinesterase and cyclooxygenase-1 activities and to allow to study their structure–function interactions with these enzymes.

Acknowledgements

We wish to thank the Algerian Ministry of High Education and Scientific Research for sponsoring this work and Mr. AMESSIS Djamel (Taghzouit, Seddouk, Bejaia city) for providing plants material.

References

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.

Adewusi, E.A., Moodley, N., Steenkamp, V., 2011. Antioxidant and acetylcholinesterase inhibitory activity of selected southern African medicinal plants. S. Afr. J. Bot. 77, 638–644.

Aidi Wannes, W., Mhamdi, B., Sriti, J., Ben Jemia, M., Ouchikh, O., Hamdaoui, G., Kchouk, M., Marzouk, B., 2010. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. Food Chem. Toxicol. 48, 1362–1370.

Amarowicz, R., Estrella, I., Teresa, H., Robredo, S., Troszyńska, A., Kosińska, A., Pegg, R.B., 2010. Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). Food Chem. 121, 705–711.

Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, P., Barl, B., Weil, J.A., 2004. Free radical-scavenging capacity, antioxidant activity of selected plant species from the Canadian prairies. Food Chem. 84, 551–562.

Berrougui, H., Isabelle, M., Cherki, M., Khalil, A., 2006. *Marrubium vulgare* extract inhibits human-LDL oxidation and enhances HDL-mediated cholesterol efflux in THP-1 macrophage. Life Sci. 80, 105–112.

Cakir, A., 2004. Essential oil and fatty acid composition of the fruits of *Hippophae rhamnoides* L. (Sea Buckthorn) and *Myrtus communis* L. from Turkey. Biochem. Syst. Ecol. 32, 809–816.

Carter, P., 1971. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Anal. Biochem. 40, 405–458.

Chryssavgi, G., Vassiliki, P., Athanasios, M., Kibouris, T., Michael, K., 2008. Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: evaluation of antioxidant capacity of methanolic extracts. Food Chem. 107, 1120–1130.

Copeland, R.A., Williams, J.M., Giannaras, J., Nurnberg, S., Covington, M., Pinto, D., Pick, S., Trzaskos, J.M., 1994. Mechanism of selective inhibition of the inducible isoform of prostaglandin G/H synthase. Proc. Natl. Acad. Sci. U.S.A. 91, 11202–11206.

Dannhardt, G., Kiefer, W., Krämer, G., Maehrlein, S., Nowe, U., Fiebich, B., 2000. The pyrrole moiety as a template for Cox-1/Cox-2 inhibitors. Eur. J. Med. Chem. 35, 499–510.

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 lemon balm (*Melissa officinalis* L.) extract. LWT 41, 391–400.

Djeridane, A., Yousfi, M., Brunel, J.M., Stocker, P., 2010. Isolation and characterization of a new steroid derivative as a powerful antioxidant from *Cleome arabica* in screening the *in vitro* antioxidant capacity of 18 Algerian medicinal plants. Food Chem. Toxicol. 48, 2599–2606.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N., 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 97, 654–660.

Dussossoy, E., Brat, P., Bony, E., Boudard, F., Poucheret, P., Mertz, C., Giaimis, J., Michel, A., 2011. Characterization, anti-oxidative and anti-inflammatory effects of Costa Rican noni juice (*Morinda citrifolia* L.). J. Ethnopharmacol. 133, 108–115.

Es-Safi, N., Khlifi, S., Kolimann, A., Kerhoas, L., El-Abbouyi, A., Ducrot, P.H., 2006. Iridoid glucosides from the aerial parts of *Globularia alypum* L. (Globulariaceae). Chem. Pharm. Bull. 54 (1), 85–88.

Fawole, O.A., Amoo, S.O., Ndhlala, A.R., Light, M.E., Finnie, J.F., Van Staden, J., 2010. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. J. Ethnopharmacol. 127, 235–241.

Fawole, O.A., Ndhlala, A.R., Amoo, S.O., Finnie, J.F., Van Staden, J., 2009. Anti-inflammatory and phytochemical properties of twelve medicinal plants used for treating gastro-intestinal ailments in South Africa. J. Ethnopharmacol. 123, 237–243.

Ferreira, A., Proença, C., Serralheiro, M.L.M., Araújo, M.E.M., 2006. The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. J. Ethnopharmacol. 108, 31–37.

Fiorucci, S., Meli, R., Bucci, M., Cirino, G., 2001. Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy? Biol. Pharmacol. 62, 1433–1438.

Fratianni, F., Tucci, M., De Palma, M., Pepe, R., Nazzaro, F., 2007. Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). Food Chem. 104, 1282–1286.

Galvez, C.J., Martin-Cordero, P., Houghton, A.M., 2005. Antioxidant activity of methanol extracts obtained from Plantago species. J. Agric. Food Chem. 53, 1927–1933.

Gibson, G.E., Huang, H.M., 2005. Oxidative stress in Alzheimer's disease. Neurobiol. Aging 26, 575–578.

Gierse, J., Nickols, M., Leahy, K., Warner, J., Zhang, Y., Cortes-Burgos, L., Carter, J., Seibert, K., Masferrer, J., 2008. Evaluation of Cox-1/Cox-2 selectivity and potency of a new class of Cox-2 inhibitors. Eur. J. Pharmacol. 588, 93–98.

Hagerman, A.E., Butler, L.G., 1978. Protein precipitation method for the quantitative determination of tannins. J. Agric. Food Chem. 26 (4), 809–812.

Heinrich, M., Teoh, H.L., 2004. Galanthamine from snowdrop – the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. J. Ethnopharmacol. 92, 147–162.

Hernandez, M.F., Falé, P.L.V., Araújo, M.E.M., Serralheiro, M.L.M., 2010. Acetylcholinesterase inhibition and antioxidant activity of the water extracts of several *Hypericum* species. Food Chem. 120, 1076–1082.

Hostettmann, K., Borloz, A., Urbain, A., Marston, A., 2006. Natural product inhibitors of acetylcholinesterase. Curr. Org. Chem. 10, 825–847.

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.

Katalinic, V., Milos, M., Kulisic, T., Jukic, M., 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 94, 550–557.

Khadri, A., Neffati, M., Smiti, S., Falé, P., Lino, A.R.L., Serralheiro, M.L.M., Araújo, M.E.M., 2010. Antioxidant, antiacetylcholinesterase and antimicrobial activities of *Cymbopogon schoenanthus* L. Spreng (lemon grass) from Tunisia. LWT Food Sci. Technol. 43, 331–336.

Loïzzo, M.R., Tundis, R., Conforti, F., Menichini, F., Bonesi, M., Nadjafi, F., Giuseppe Frega, N., Menichini, F., 2010. *Salvia leriifolia* Benth (Lamiaceae) extract demonstrates *in vitro* antioxidant properties and cholinesterase inhibitory activity. Nutr. Res. 30, 823–830.

Maksimovic, Z., Malencic, D., Kovacevic, N., 2005. Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. Bioresour. Technol. 96, 873–877.

Mukherjee, P.K., Kumar, V., Mal, M., Houghton, P.J., 2007. Acetylcholinesterase inhibitors from plants. Phytomedicine 14, 289–300.

Orhan, I., Sener, B., Choudhary, M.I., Khalid, A., 2004. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. J. Ethnopharmacol. 91, 57–60.

Orhan, I., Şenol, F.S., Gülpinar, A.R., Kartal, M., Şekeroglu, N., Deveci, M., Kan, Y., Şener, B., 2009. Acetylcholinesterase inhibitory and antioxidant properties of *Cyclotrichium niveum*, *Thymus praecox* subsp. *caucasicus* var. *caucasicus*, *Echinacea purpurea* and *E. pallida*. Food Chem. Toxicol. 47, 1304–1310.

Ozsoy, N., Can, A., Yanardag, R., Akev, N., 2008. Antioxidant activity of *Smilax excels* L. leaf extracts. Food Chem. 110, 571–583.

Quezel, P., Santa, S., 1963. Nouvelle flore de l'Algérie et des régions désertiques et méridionales, Tome II. Edition CNRS, Paris, pp. 611–1170.

Rapisarda, P., Tomaino, A., Lo Cascio, R., Bonina, F., De Pasquale, A., Saija, A., 1999. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. J. Agric. Food Chem. 47, 4718–4723.

Rodríguez-Pérez, C., Quirantes-Piné, R., Amessis-Ouchemoukh, N., Madani, K., Segura-Carretero, A., Fernández-Gutierrez, A., 2013. A metabolite-profiling approach allows the identification of new compounds from *Pistacia lentiscus* leaves. J. Pharm. Biomed. Anal. 77, 167–174.

Romani, A., Pinelli, P., Galardi, C., Mulinacci, N., Tattini, M., 2002. Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of *Pistacia lentiscus* L. Phytochem. Anal. 13 (2), 79–86.

Sahpaz, S., Garbacki, N., Tits, M., Bailleul, F., 2002. Isolation and pharmacological activity of phenylpropanoid esters from *Marrubium vulgare*. J. Ethnopharmacol. 79, 389–392.

Schulz, V., 2003. Ginkgo extract or cholinesterase inhibitors in patients with dementia: what clinical trial and guidelines fail to consider. Phytomedicine 10, 74–79.

Shyu, Y.S., Lin, J.T., Chang, Y.T., Chiang, C.J., Yang, D.J., 2009. Evaluation of antioxidant ability of ethanolic extract from dill (*Anethum graveolens* L.) flower. Food Chem. 115, 515–521.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acids reagents. Am. J. Enol. Vitic. 16, 144–158.

Taskova, R.M., Gotfredsen, C.H., Jensen, R.S., 2006. Chemotaxonomy of Veroniceae and its allies in the Plantaginaceae. Phytochemistry 67, 286–301.

Ulbrich, H., Fiebich, B., Dannhardt, G., 2002. Cyclooxygenase-1/2 (Cox-1/Cox-2) and 5-lipoxygenase (5-LOX) inhibitors of the 6,7-diaryl-2,3-1H-dihydropyrrolizine type. Eur. J. Med. Chem. 37, 953–959.

Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46, 4113–4117.

Vonkeman, H.E., van de Laar, M.A.F.J., 2010. Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention. Semin. Arthritis Rheum. 39, 294–312.