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Chemicals profiling and antioxidants activities of *Acacia* seeds

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This study reported investigations on phytochemical screening and antioxidants activities of seeds from seven *Acacia* species. Storage proteins and mineral contents were determined. The seed extracts of *Acacia* species were evaluated for their total phenols, flavonoids, carotenoids contents and total antioxidant capacity assessed by 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1- picrylhydrazyl (DPPH) assays. Total protein content ranged from 99.49 (*Acacia cyclops*) to 142.77 (*Acacia cyanaphylla*) mg/g dry weight basis (DW) according to the *Acacia* species, having an average of 120.41 mg/g DW. The albumin constitutes the major fraction presenting 31.11%, followed by globulin (27.36%), prolamin (22.27%) and glutelin (19.27%). The *Acacia* seeds were rich in minerals. The total phenolic compounds (TPC) determinate ranged from 154.47 (*A. cyclops*) to 1217.99 (*Acacia horrida*) with an average of 632.40 mg gallic acid equivalents/100 g DW. The total flavonoid contents have an average of 11.21 mg rutin equivalents/100 g DW, and the carotenoids mean is 4.23 mg/kg. The *Acacia* seed extracts have an antioxidant activity reflected by the ABTS and DPPH tests. The Duncan's test showed significant difference of proteinic, phenolics, flavonoid and carotenoid contents according to the *Acacia* species. Therefore, natural components from *Acacia* seeds could be incorporated as food ingredient, without causing detrimental effects to the food's palatability and the functional ingredient's efficacy.

Key words: *Acacia* seeds, storage proteins, minerals, polyphenols, flavonoids, radical scavenging activity.

INTRODUCTION

Recently, there is a rising interest of natural antioxidants as currently used synthetic antioxidants have been suspected to cause or promote undesirable effects on human health. Research related to antioxidants represents nearly 70% of the studies used on functional foods today. Most of these studies involve natural ducts such as fruits, vegetables, whole grains, herbs and leaves. Plants are potential sources of natural antioxidants because they contain phenolic compounds such as phenolic acids, flavonoids, tannins and phenolic diterpenes (Shahidi, 1997); some of them for example, tocopherols (vitamin E), ascorbic acid (vitamin C) and carotenoids are substances of major importance in human composition and food.

The large scale accessibility of agricultural and Industrial plant waste materials and their low cost makes them a striking source of natural antioxidants (Perez-Bonilla et al., 2006). Over the last years, researchers have become more and more interested in nutritional phenolic compounds because of their free radical scavenging activity and other potential beneficial effects on human health (Manach et al., 2004). Natural products based drugs have been used against various diseases since time

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Table 1. Seed morphological traits and data of *Acacia* species used in this study for chemicals profiling.

Species	Latitude	Longitude	Length (mm)	Width (mm)	Weight 100 seeds (g)	Color
A. farnesiana	33°53'N	10°06'E	9.827 ± 3.454 [A]	5.212 ± 0.428[A]	8.181 ± 2.597[A]	Brown
A. tortilis	34°25'N	08°47'E	6.466 ± 0.715 [A]	4.770 ± 0.555[B]	6.149 ± 0.654[B]	Brown
A. mollissima	35°17'N	10°41'E	4.254 ± 0.397 [A]	2.898 ± 0.536[D]	1.428 ± 0.121[E]	Black
A. cyanophylla	34°42'N	8°78'E	9.313 ± 1.75 [A]	2.438 ± 0.290[E]	1.732 ± 0.711[E]	Dark brown
A. horrida	36°36'N	08°58'E	5.527 ± 0.796 [A]	3.938 ± 0.290[C]	3.342 ± 0.213[D]	Greenish
A. salicina	33°21'N	10°30'E	9.740 ± 7.337 [A]	3.978 ± 1.656[C]	4.430 ± 0.430[C]	Dark brown
A. cyclops	33°44'N	10°45'E	5.127 ± 0.357 [A]	4.552 ± 0.780[B]	4.579 ± 1.859[C]	Dark brown

(*) Each value in the table is represented as mean ± SD (n, 30). (**) Superscript letters with different letters in the same column of cultivar, respectively indicate significant difference ($p < 0.05$) analyzed by Duncan's multiple range test.

immemorial and are a significant part of today's drugs. Folk-lore used herbs as therapeutics and medicaments. The importance of some plants has long been published but a large number of them remain unknown yet. So, it is essential to explore their uses and to conduct pharmacognostics and pharmacological studies to discover their therapeutic uses.

Recently, many flavonoids have been isolated and identified from various plants, including fruits (Havsteen, 2002). In fact, the beneficial health effects of fruits are generally attributed to flavonoids. It has been found that these bioactive compounds possess a high effect to reduce several diseases such as cardiovascular, neurodegenerative and lung cancers (Ozgen et al., 2008; Shen et al., 2009). Since ancient times, several diseases have been treated by administration of plant extracts based on traditional medicine (Pezzoto, 1997). Investigation of traditionally used medicinal plants is thus, valuable on two levels; firstly, as a source of potential chemotherapeutic drugs, and secondly, as a measure of safety for the continued use of medicinal plants. In fact, plants and microorganisms provide the pharmaceutical industry with some of the most important sources of components for the research of new medications.

Research has increased to investigate new natural sources of antioxidants, especially from underexploited plants. The genus *Acacia* comprises approximately 1350 species (Maslin et al., 2003), widespread in the warm sub-arid and arid parts of the world. It has been reported that various *Acacia* species were rich in secondary metabolites such as alkaloids, cyanogenic glycosides, cyclitols, gums, terpenes, flavonoids and condensed tannins (Maslin et al., 2003). *Acacia* is used in traditional medicine as an antibacterian, antioxidant, anti-inflammatory, astringent, antispasmodic and anti-arythmic agent (El Abbouyi et al., 2004).

Despite the occurrence of some published preliminary work describing the phytochemical contents of the *Acacia* leaf in Tunisia (Bouhlel et al., 2007), little is known about the phytochemicals of *Acacia* seeds. Owing to the role of antioxidants in the indicated medicinal and nutritional uses of the plant, our present study was designed to isolate the antioxidant principles, proteins and their

fraction constituents from seeds of seven Tunisian *Acacia* species. The antioxidant capacity of *Acacia* seed extracts were evaluated using radical scavenging activity of 2,2-diphenyl-1- picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) tests.

MATERIALS AND METHODS

Plant materials

Seeds were randomly picked from at least three trees of each *Acacia* species. Samples from fully mature fruits were included in the present study. *Acacia* seeds came from seven species located in Tunisia. Thirty (30) seeds were sampled on each *Acacia* species. Three pomological parameters were determined; length, width and weight of 100 seeds. Each parameter value is represented as mean ± standard deviation (n, 30). The colors of *Acacia* seeds were also evaluated. Table 1 gives more information about their geographical origin and their seed pomology.

Chemicals

All solvents used were of reagent grade without any further purification. Gallic acid, rutin and Folin-Ciocalteu's phenol reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DPPH and ABTS were purchased from Sigma Chemical Co. (Poole, Dorset, UK). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Fluka Chemical Co. (Ronkonkoma, NY, USA). The analytical reagent grade methanol was obtained from Lab-Scan (Labscan Ltd, Dublin, Ireland). The water used in sampling was prepared with a Millipore Simplicity (Millipore S.A.S., Molsheim, France). Spectrophotometric measurements were performed on Shimadzu UV-1600 spectrometer (Shimadzu, Kyoto, Japan).

Storage proteins extraction from *Acacia* seed

In order to extract all classes of storage proteins of *Acacia* seeds, we adopted a fractionation protocol of the various categories of proteins based on their solubility differences as described previously by Nasri and Triki (2007) and later by Elfalleh et al. (2010). The extraction procedure is based on solubility differences of proteins in various solvents (Osborn, 1924). First, a milled sample (500 mg dry weight (DW) of delipided seed) was extracted with distilled water (10 ml). The suspension was stirred at laboratory temperature for 20 min and then centrifuged at 10000 rpm for 15 min. The filtrated supernatant was used as the

extract 1 (albumin fraction). The remaining insoluble sample was mixed with 10 ml of aqueous NaCl 5% (w/v) solution. The extraction procedure was repeated, and the extract 2 was collected (globulin fraction). After following extractions with aqueous 70% (v/v) ethanol and aqueous 0.2% NaOH solution, the extract 3 (prolamin fraction) and extract 4 (glutelin fraction) were obtained.

Storage protein quantification using Bradford assay

The protein content of each sample was quantified using method according to Bradford (1976). 100 mg Coomassie Brilliant Blue G-250 (Sigma-Adrich Co) was dissolved in 50 ml ethanol (95%) and 100 ml of phosphoric acid (85%) was added. The solution was diluted, filtered and used as the color reagent for protein quantification. Standard solutions of reagent grade BSA (Equitech-Bio, Inc., Kerriville, TX) were prepared containing 0 to 400 µg proteins. Samples were covered with parafilm mixed and then incubated for 5 min before absorbance measurement at 595 nm in spectrophotometer (Anthelie Advanced, Microbeam, and S.A). All standard and unknown samples possessed the same solution matrix. The protein content of each sample was determined by fitting a least squares regression curve of the quantity of standard protein concentration versus photometric absorbance.

Minerals contents

Minerals were quantified in triplicate as previously described (Elfalleh et al., 2009). Plant material was dried at 70°C. One gram (1 g) of powdered form of the sample was placed in a porcelain crucible in a muffle fumace at a temperature of 550°C. After cooling, the ash was suspended in 5 ml of deionised water and 1 ml of hydrochloric acid; and was autoclaved. The crucible contents were then filtered and diluted to 100 ml with water. The resulting solution was used for mineral analysis. The combined concentration of calcium and magnesium ions was determined by complexometric titration (Ringbom et al., 1958). The sodium and potassium were determined by flame photometer (Sherwood 410, Sherwood Scientific Ltd, Cambridge, UK). Total phosphorus was determined by a spectrophotometer (Secomam S1000, Secomam, Ales and France).

Carotenoid analysis

The quantification of carotenoids as xanthophylls and carotenes entail with the determination of chlorophyll (Chl) Chla and Chlb by UV-VIS spectroscopy. Chlorophyll and carotenoids were extracted from *Acacia* seed using a method modified by Gitelson et al. (2003). Briefly, samples were put into a pre-chilled tube and ground for 3 min in 1 ml extraction buffer (80% acetone: Tris-HCl [1%, w/v]). After the pigments were completely extracted by the buffer, an additional 1 ml extraction buffer was used to wash the pestle. All extraction solutions were combined and debris was removed by centrifugation. A volume of 1 ml of the supernatant was diluted to 3 ml final solution. The light absorbance of the final solution was measured at 663, 647 and 470 nm. The concentrations of carotenoids and chlorophyll were calculated as described by Lichtenthaler (1987). All experiments were done in triplicate and the carotenoid contents were converted to mg per kg of fresh weight.

Phenolics extraction

Total phenolic compounds (TPC) were extracted from the seed powders. The seed powder (10 g) was extracted and stirred with

100 ml MeOH at 30°C for one night. The extract was filteredthrough Whatman no. 1 filter paper for removal of seed particles. The residue was re-extracted with 60 ml methanol. The obtained extracts were filtered again. *Acacia* seed extracts were pooled and concentrated under vacuum at 40°C. Obtained methanol ic extract were used for phenolic and antioxidant analyses.

Determination of total polyphenols and total flavonoids contents

The Folin-Ciocalteu method was used to measure the TPCs (Elfalleh et al., 2009). For the analysis, from each sample, 0.5 ml of methanolic extract solution was added to 0.5 ml of Folin-Ciocalteu reagent (Prolabo, Paris France), followed by 4 ml of 1 M sodium carbonate. Next, the test tubes were incubated at 45°C for 5 min and then cooled in cold water. Absorbance was measured at 765 nm, using a Shimadzu 1600-UV spectrophotometer (Shimadzu, Kyoto, Japan). The results were compared to a gallic acid calibration curve, and the TPCs were determined as mg gallic acid equivalents per 100 g DW (GAE mg/100 g DW). Determination of each sample was performed in triplicate.

Total flavonoids were measured spectrophotometrically in triplicate following the method described previously (Elfalleh et al., 2009). This method is based on the formation of a complex flavonoid–aluminium, having the maximum absorbance at 430 nm. Rutin was used to make a calibration curve. One ml (1 ml) of methanolic extract was mixed with 1 ml of 2% AlCl$_3$ methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm using a Shimadzu 1600-UV spectrophotometer. The flavonoids content was expressed as rutin equivalents in mg per 100 g DW (mg RE/100 g DW).

Antioxidant activities

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activities

The scavenging activity of methanolic extracts on DPPH radical was determined in triplicate following the method reported by Okonogi et al., (2007). A methanolic test solution of defferent concentrations was prepared from a stock solution of seed extracts (1 mg of dry powder per ml). DPPH (100 µM) was dissolved in ethanol and mixed with an aliquot of 100 µl of each dilution. After the reaction was allowed to take place in the dark for 30 min, then the absorbance at 517 nm was recorded to determine the concentration of remaining DPPH. Results were expressed as Trolox equivalent antioxidant capacity (TEAC).

The total antioxidant activity values were estimated in triplicate by TEAC. In this test, we measured the relative capacity of antioxidants to scavenge the ABTS$^{.+}$ radical compared to the antioxidant potency of Trolox which is used as a standard. The ABTS$^{.+}$ radical was generated by mixing 7 mM ABTS solution with 2.45 mM K$_2$S$_2$O$_8$ in the dark for 24 h at room temperature. Before usage, the ABTS$^{.+}$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm after 5 min. 25 µl of antioxidant sample or Trolox standard was added to 1 ml of the diluted ABTS$^{.+}$ solution. The reaction mixture was homogenised for 20 s and then the absorbance was recorded at 734 nm at 5 min. The final TEAC value of the antioxidant compound was calculated by comparing ABTS$^{.+}$ decolourisation with Trolox, which gives a useful indication of the antioxidant potential of the plant extracts. Measurement was performed in triplicate." to "All measurements were performed in triplicate.

Table 2. Seed storage protein composition (mg/g of dry weight basis of delipided seed) of *Acacia* species studied.

Species	Albumin	Globulin	Prolamin	Glutelin	Σ SSP
A. farnesiana	37.62 ± 1.05[B]	21.72 ± 1.76[E]	28.74 ± 1.71[B]	22.11 ± 0.15[C]	110.19 ± 1.00[C]
A. tortilis	26.82 ± 0.99[D]	31.59 ± 1.73[C]	33.62 ± 2.13[A]	19.44 ± 0.09[D]	111.47 ± 1.10[C]
A. mollissima	53.40 ± 1.97[A]	16.56 ± 0.99[F]	28.05 ± 0.50[B]	25.38 ± 2.00[AB]	123.39 ± 4.21[B]
A. cyanophylla	33.78 ± 2.94[C]	55.71 ± 1.99[A]	29.40 ± 1.94[B]	23.88 ± 1.00[BC]	142.77 ± 5.79[A]
A. horrida	26.91 ± 2.06[D]	28.14 ± 1.02[D]	31.29 ± 1.73[AB]	26.58 ± 0.93[A]	112.92 ± 2.17[C]
A. salicina	54.06 ± 2.00[A]	40.08 ± 2.02[B]	29.09 ± 0.81[B]	19.44 ± 0.09[D]	142.67 ± 2.79[A]
A. cyclops	29.61 ± 2.99[D]	36.80 ± 3.15[B]	7.52 ± 4.16[C]	25.56 ± 2.00[AB]	99.49 ± 1.23[D]
Mean	37.46 ± 11.76	32.94 ± 12.93	26.82 ± 8.72	23.20 ± 2.93	120.41 ± 16.75
Proteinic fraction (%)	31.11	27.36	22.27	19.27	12

(*) Each value in the table is represented as mean ± SD (n, 3). (**) Superscript letters with different letters in the same column of cultivar, respectively indicate significant difference ($p < 0.05$) analyzed by Duncan's multiple range test.

Statistical and chemometric methods

The results of compound contents were reported as mean values of three replicates (mean ± standard deviation, n, 3). Data were compared on the basis of standard deviation of the mean values. Differences between mean values were assessed based on a one-way analysis of variance with a post-hoc determination using Duncan's multiple range tests and using Statistical software (version 8). The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Proteins content of *Acacia* seeds

The contents of storage proteins extracted from *Acacia* seeds have an average of 120.41 mg/g DW basis of delipided seed (Table 2). The two *Acacia* species *cyanophylla* and *silicia* have the highest protein contents, respectively 142.77 and 142.67 mg/g DW; whereas, the *Acacia cyclops* has the lowest protein content (99.49 mg/g DW). The albumin is the major fraction (31.11%), followed by globulin (27.36%), prolamin (22.27%) and glutelin (19.27%) fractions. The Duncan's test showed significant differences between protein fractions of *Acacia* species. Ash content ranged from 2.10 (*Acacia farnesiana*) to 6.00% (*Acacia cyanophylla*). The variation of ash content did not showed a significant difference between *Acacia* species (Table 3).

The seed storage proteins are important because they ensure feeding the germinating embryo which enables it to attain the autotrophy. They are also important for the human and animal nutrition providing more than the half of daily protein requirement (Cheftel et al., 1985). Most of the physiologically active proteins (enzymes) are found in the albumin or globulin groups. Nutritionally, the albumins and globulins have a very good amino acid balance. Globulins, prolamins and glutelins as storage reserves are not present systematically in the seeds of all plant species (Bewley and Black, 1983). In the present study, four fractions (albumine, globuline, prolamin and glutelin) constitute the *Acacia* seeds protein (Table 2).

The differences between *Acacia* species suggested that the genotype is the major factor influencing the protein fractions constituents which are in agreement with those for most legumes (Vasconcelos et al., 2010). However, it has been reported that protein fractions varied according to several factors as in *vignera* genus, the extracting method, the cultivars and also the genetic and environmental factors (Adsule et al., 1986). Proteins from *Acacia* seeds are relatively abundant (12%), and should be investigated further. Storage chemicals from seeds of non-conventional plants should be explored. Proteins, for example, play significant roles in human health. Mainly in developing countries where the average protein intake is less than required, it is essential to find new sources of edible protein and other nutrients to overcome the population problem.

Mineral contents of *Acacia* seeds

The mineral content of *Acacia* seeds was expressed as mg per 100 g DW basis (Table 3). Potassium is the most abundant mineral in dry *Acacia* seeds with an average of 530.07 mg/100 g DW, followed by the calcium and magnesium levels having 195.28 mg/100 g DW. The mean of phosphorus and sodium contents were, respectively 36.34 and 10.84 mg/100 g DW (Table 3).

Duncan's test showed significant differences on the mineral contents of *Acacia* species. It has been reported that variation on mineral content in the leguminous plants could be related to genetic origin, geographical source and the level of soil fertility (Apata and Ologhobo, 1994).

Antioxidants contents of *Acacia* seeds

The total phenolics levels have an average of 632.40 mg GAE/100 g DW (Table 4). It varied from 154.47 (*A. cyclops*) to 1217.99 mg GAE/100 g DW (*Acacia horrida*). The total flavonoid contents ranged from 7.10 (*Acacia tortilis*) to 29.35 mg GAE/100g DW (*Acacia cyanophylla*).

Table 3. Mineral contents* (mg per 100 g dry weight basis) from seeds of *Acacia* species studied.

Species	Ash (%)	Phosphorus	Potassium	Sodium	Calcium and Magnesium
A. farnesiana	2.10 ± 0.42[A]	21.97 ± 1.41[CD]	276.00 ± 31.11[EF]	4.05 ± 1.41[C]	135.56 ± 21.21[C]
A. tortilis	4.41 ± 0.56[A]	31.75 ± 4.24[C]	236.57 ± 28.28[F]	9.11 ± 2.83[BC]	508.88 ± 141.42[A]
A. mollissima	4.00 ± 1.41[A]	20.18 ± 5.66[D]	389.29 ± 77.78[DE]	12.14 ± 2.83[AB]	126.11 ± 21.21[C]
A. cyanophylla	6.00 ± 1.41[A]	84.76 ± 0.78[A]	492.86 ± 28.28[D]	11.13 ± 1.41[AB]	73.33 ± 2.83[D]
A. horrida	4.35 ± 1.41[A]	44.68 ± 4.24[B]	778.01 ± 70.71[B]	8.10 ± 2.83[BC]	151.17 ± 3.54[C]
A. salicina	4.40 ± 2.83[A]	25.31 ± 4.24[CD]	630.86 ± 42.43[C]	13.16 ± 2.83[AB]	250.83 ± 0.78[BC]
A. cyclops	4.80 ± 1.41[A]	25.71 ± 4.95[CD]	906.87 ± 70.71[A]	18.22 ± 5.66[A]	121.11 ± 28.28[C]
Mean	4.29 ± 1.16	36.34 ± 22.87	530.07 ± 253.48	10.84 ± 4.44	195.28 ± 148.38

(*) Each value in the table is represented as mean ± SD (n, 3). (**) Superscript letters with different letters in the same column of cultivar, respectively indicate significant difference ($p < 0.05$) analyzed by Duncan's multiple range test.

Table 4. Phytochemical and antioxidant screening of seeds of *Acacia* species studied.

Species	Total phenolics (mg GAE/100 g DW)	Total flavonoids (mg RE/100 g DW)	Carotenoids (mg/kg)	DPPH (TEAC mM)	ABTS (TEAC mM)
A. farnesiana	275.98 ± 20.00[E]	12.10 ± 1.73[BC]	1.20 ± 0.18[C]	0.78 ± 0.06[A]	2.45 ± 0.20[A]
A. tortilis	536.77 ± 26.46[D]	7.10 ± 2.00[D]	6.71 ± 1.00[A]	0.84 ± 0.03[A]	2.22 ± 0.20[A]
A. mollissima	718.91 ± 81.15[BC]	9.08 ± 0.79[CD]	3.28 ± 0.49[B]	0.84 ± 0.10[A]	0.90 ± 0.09[C]
A. cyanophylla	190.80 ± 15.67[E]	29.35 ± 2.00[A]	6.21 ± 0.93[A]	0.88 ± 0.07[A]	1.29 ± 0.18[B]
A. horrida	1217.99 ± 200.00[A]	12.84 ± 2.27[B]	4.23 ± 0.63[B]	0.34 ± 0.03[B]	2.25 ± 0.20[A]
A. salicina	650.92 ± 40.00[CD]	12.61 ± 1.65[B]	1.25 ± 0.19[C]	0.32 ± 0.02[B]	0.66 ± 0.20[C]
A. cyclops	154.47 ± 32.95[E]	11.39 ± 2.08[BC]	6.71 ± 1.00[A]	0.33 ± 0.06[B]	1.24 ± 0.24[B]
Mean	632.40 ± 358.85	11.21 ± 2.29	4.23 ± 2.42	0.62 ± 0.27	1.57 ± 0.72

(*) Each value in the table is represented as mean ± SD (n, 3). (**) Superscript letters with different letters in the same column of cultivar respectively indicate significant difference ($p < 0.05$) analyzed by Duncan's multiple range test.

Little information is available for antioxidants from *Acacia* seeds. As for *Acacia salicina* leaves, phenolic contents were ca.180 µg GAE/g DW (Chatti et al., 2009).

Phenolic compounds of more than 8000 known, embrace a wide range of plants secondary metabolites (Harborne, 1994; Pietta, 2000). Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom. Flavonoids constitute the largest class of phenolic compounds with more than 5000 structures, possessing in common a flavylium unit (C6-C3-C6) (Iacobucci and Sweeny, 1983).

Phenolics play an important role in plant metabolism, but also protect the plant against stresses. For instance, it has been recently shown that flavonoids, such as catechin, regulate the auxin transport in plants, and therefore, play an important role in plant development (Brown et al., 2001). Several studies have shown that the plant resistance to both biotic (pathogens and predators) and abiotic (UV-radiation, drought, etc.) stresses is related to phenolic compounds (Parr and Bolwell, 2000; Dicko et al., 2005). All classes of phenolic compounds (hydroxybenzoic acids, hydroxycinnamic acid derivatives, flavonoids, polyflavans, etc.) are involved in the resistance mechanisms.

A number of highly reactive oxygen species such as singlet oxygen ($1O_2$), superoxide anion radical ($O_2•-$), hydroxyl radical (OH•), nitric oxide radical (NO•) and alkyl peroxyl (ROO•) are regularly produced in the human body (Langseth, 1995). These radicals can damage lipids, proteins and DNA and participate in pathogenesis and ageing (Ryan and Robards, 1998; Santos-Buelga and Scalbert, 2000; Parr and Bolwell, 2000). Phenolic compounds together with other natural compounds (vitamins C and E, and carotenoids), contribute to the defense by scavenging free radicals, inhibiting oxidative enzymes such as lipooxygenase and cyclooxygenase and chelating metal ions (Shi et al., 2001).

The antioxidant activities of the extracts from *Acacia* seeds were evaluated as TEAC calculated from DPPH•- and ABTS•+ scavenging capacity (Table 4).

The free radical scavenging activity determined by DPPH varied from 0.32 (*Acacia salicina*) to 0.88 mM TEAC (*A. cyanophylla*) with an average of 0.62 mM TEAC in *Acacia* seeds extract. The values determined by ABTS ranged from 0.66 (*A. salicina*) to 2.45 mM TEAC (*Acacia farnesiana*), with an average of 1.57 mM TEAC.

Free radical scavenging activity determined by DPPH and ABTS were usually used in several plant extracts

(Elfalleh et al., 2009; Nasri et al., 2011).

Considering that multiple reaction characteristics and mechanisms involved in the estimation of the total antioxidants, no single method could accurately reflect all the antioxidants in a mixed system due to the complex nature of phytochemicals (Chu et al., 2000). Currently, the ABTS method was used to confirm the results obtained on the DPPH test since both methods are based on a similar antioxidant mechanism and the extracts used in both tests were methanol-soluble.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals (Bors et al., 2001). In addition, synergism between the antioxidants in the mixture makes the antioxidant capacity not only dependant on the concentration, but also on the structure and the interaction between the antioxidants (Djeridane et al., 2006).

Antioxidant activities of phenolic compounds have been suggested to exert beneficial pharmacological effects on neurological disorders on the basis of *in vitro* observations (Moosmann and Behl, 1999).

Conclusion

These results obtained show that the unexploited *Acacia* seeds seem to be a new interesting natural source of protein, minerals and antioxidants. The *Acacia* seed extracts showed antioxidant activities reflected by ABTS and DPPH tests. Therefore, consumption of food produced with natural compounds extracts (functional foods) is expected to prevent the risk of free radical dependent diseases. The use of investigated natural resources spices such as the unexploited *Acacia* seeds could be useful not only in food and cosmetics production but also as important functional food in the prevention and treatment of various human diseases. The development of a functional food is a long and complex process, which requires multidisciplinary approaches. *Acacia* seed used for functional foods and dietary supplements is still in the development stage requiring continuing research.

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