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Revalorization of wild *Asparagus stipularis* Forssk. as a traditional vegetable with nutritional and functional properties

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The nutritional values and phytochemical composition of young shoots of wild *Asparagus stipularis* Forssk. from Tunisia were determined in this study. The antioxidant (tested by four different *in vitro* assays) and antiproliferative properties (against human tumour cell lines) of aqueous and hydroalcoholic extracts of the young shoots were also investigated. The obtained results indicated that the young shoots are a potential source of valuable nutrients and dietary fiber, and their hydroalcoholic extract showed marked antioxidant and antiproliferative activities in all tested assays. The major phenolic compound, characterized by using a high-performance liquid chromatograph coupled with diode-array and mass spectrometer detectors, in both extracts was diferuloyl glycerol. Our results reinforce the interest in recovering the traditional culinary uses of this wild plant, with potential functional properties, which can be included in the modern diet as an alternative to the variety of vegetables normally used.

1. Introduction

Asparagus is a genus in the Liliaceae family that includes over 250 species.[1] Although *Asparagus officinalis* L. is the only cultivated asparagus nowadays, other wild species are edible and traditionally consumed in Europe and the Mediterranean Basin, such as *Asparagus acutifolius* L. and *Asparagus stipularis* Forssk. Previous studies showed that wild *Asparagus*, particularly *Asparagus acutifolius*, is a good source of valuable nutrients including essential minerals, dietary fibers, vitamins and amino acids.[1–7] In addition, *Asparagus* species are rich in bioactive components, mainly phenolic compounds, responsible for their functional properties.[7–9] In particular, the medicinal properties of this genus have been described in both European and Asian cultures.[10,11]

Young shoots of wild *Asparagus stipularis* are traditionally consumed by rural populations of Cyprus, Rhodes, Greece, Egypt, Algeria and Tunisia.[12] In addition, the infusion of their tuberous roots has been reported to alleviate headache, prevent renal stone formation and cure syphilis. The decoction of the whole plant is used to relieve stomach ache and promote appetite.[13] Although the phytochemistry and phytotherapy of cultivated *Asparagus officinalis* and wild *Asparagus acutifolius* are relatively well known, there have been very few published data on the biochemical composition and biological properties of *A. stipularis* young shoots.

Wild edible food sources, particularly wild vegetables, have fallen into neglect since their traditional uses are being lost throughout generations. The revalorization of these wild vegetables as potential sources of functional food ingredients and/or including their consumption in modern diets could contribute to the preservation of their traditional knowledge and culinary uses, as well as to improve the biodiversity and rural sustainability of the collection areas. Thus, the aim of this study was to characterize the nutritional value and bioactive compounds of *A. stipularis* young shoots, paying special attention to the functional properties (antioxidant and antiproliferative activities) of the aqueous and hydroalcoholic extracts.

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2. Materials and methods

2.1. Plant material

Wild *Asparagus stipularis* Forssk. young shoots were collected in January (2015) from the cliff of Monastir (Tunisia). The plant was dried by active ventilation at 37 °C. The dried young shoots were ground in an ultra-centrifugal mill ZM 200 (Retsch, Haan, Germany).

2.2. Standards and reagents

All organic solvents were HPLC grade. HMDS, TMCS, pyridine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). The metals (Fe, Cu, Mn and Zn) were purchased from Merck (Darmstadt, Germany). The standards of organic acids (citric acid, malic acid and oxalic acid) and tocopherols (α-, β-, γ-, and δ-isoforms) were purchased from Sigma. The phenolic compound standards were obtained from Extrasynthese (Genay, France).

2.3. Nutritional value

2.3.1. Proximate composition. Moisture, protein, fat and ash were determined following the AOAC procedures.[14] The moisture content was determined using an air oven. The crude fat was extracted using a Soxhlet apparatus. The nitrogen content was estimated by using the micro-Kjeldahl method and the factor 6.25 was used to convert the nitrogen content to protein content. The ash content was determined by incineration at 550 ± 15 °C.

2.3.2. Carbohydrates (soluble sugars, oligosaccharides and polyols). The extraction, identification and characterization of individual soluble sugars were done by GS-MS according to the method described by Mechri *et al.*[15] The quantification of each compound was performed using the internal standard calculation method. The analyses were performed in triplicate. The values are expressed as mg per 100 g fw.

2.3.3. Total, soluble and insoluble dietary fiber. The fiber content was determined according to the enzymatic-gravimetric methods (993.19 and 991.42) as previously described.[16] Powdered spear was subjected to sequential enzymatic digestion by α-amylase, protease and amyloglucosidase. Vacuum filtration was used to separate the soluble and insoluble fractions. The residue of filtration was dried at 100 °C and the ash and protein contents were determined. The contents of total dietary fibre as well as soluble and insoluble dietary fibre fractions are expressed as g per 100 g fw sample.

2.3.4. Energy. Energy was calculated according to the following equation:

$$\text{Energy (kcal per 100 g fresh weight)} = [4 \times (g_{\text{protein}} + g_{\text{soluble sugar}}) + 2 \times (g_{\text{fiber}}) + 9 \times (g_{\text{fat}})].$$

2.3.5. Mineral elements. The method 930.05 of the AOAC[14] procedures was used. 300 mg of powdered young shoots was subjected to dry-ash mineralization in an oven at 550 ± 15 °C. The residue was digested with HCl (50% v/v) and HNO_3 (50% v/v). Zinc (Zn), iron (Fe), copper (Cu) and magnesium (Mn) were quantified by atomic absorption spectroscopy (AAS) using an Analyst 200 PerkinElmer equipment (PerkinElmer, Waltham, MA, USA). An additional 1/10 (v/v) dilution of the sample fraction and standards was performed for macro-element determination: for Ca and Mg analysis in 1.16% La_2O_3/HCl (leading to $LaCl_2$) and for Na and K analysis in 0.2% CsCl. The results are expressed in mg per 100 g fw.

2.3.6. Fatty acid composition. Fatty acids were determined by using the gas–liquid chromatographic method[17] with slight modifications. Fatty acids (obtained after Soxhlet extraction) were treated with methanolic KOH to obtain fatty acid methyl esters (FAMEs). FAMEs were identified by comparison of their retention time with the pure standards analyzed under the same conditions. All fatty acid peak areas were calculated using the HP ChemStation software and recorded as peak area percentages. The results are expressed as relative percentage.

2.4. Bioactive compounds

2.4.1. Organic acids. Organic acids were determined using ultra-fast liquid chromatography (UFLC) following a procedure previously optimized and described by Dias *et al.*[18] The organic acids found were quantified by comparison of the area of their peaks recorded with the calibration curves obtained from the commercial standards of each compound. The results are expressed in g per 100 g of fresh weight (fw).

2.4.2. Tocopherols. Tocopherols were determined following a procedure previously described by Dias *et al.*[18] The compounds were identified by chromatographic comparison with authentic standards. The quantification was based on the fluorescence signal response of each standard, using the internal standard method. The results are expressed in mg per 100 g of fresh weight (fw).

2.4.3. Volatile compound analysis. Solid-phase microextraction (SPME) was performed with further analysis by GC-MS as previously described.[19] The retention times served for the identification of constituents comparing their linear retention indices (LRI) on computer matching against the library mass spectra, and the MS literature data.

2.4.4. Phenolic compounds

Preparation of aqueous and hydroalcoholic extracts. The extracts were prepared as previously described.[13] For hydroalcoholic extraction, 1 g of powdered young shoots was stirred with 30 mL of a mixture of methanol : water (80 : 20, v/v) at 25 °C and 150 rpm for 1 h. The extract was then filtered and the residue was further extracted with a 30 mL portion of the hydroalcoholic mixture. The combined supernatants were then evaporated and lyophilized. For aqueous preparation (infusion), 1 g of powdered young shoots was added to 200 ml of boiled water and allowed to stabilize at room temperature. The solution was then filtered, frozen and lyophilized.

HPLC-DAD-ESI/MS analyses for individual phenolic compounds. The analyses of individual phenolic compounds were carried out on the hydroalcoholic and infusion extracts by HPLC using double online detection by diode array spectrophotometry and mass spectrometry (MS). A Hewlett-Packard

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1100 chromatograph (Hewlett-Packard 1100, Agilent Technologies, Santa Clara, CA, USA) provided with a quaternary pump and a diode array detector (DAD) coupled with an HP ChemStation (rev. A.05.04) data-processing station was used. The system was connected *via* the cell outlet to an MS detector API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) that was controlled by the Analyst 5.1 software. The separation was achieved on an Agilent Poroshell 120 EC-C18, 2.7 μm (4.6 × 150 mm) column thermostated at 35 °C. The solvents were (A) 0.1% formic acid and (B) acetonitrile. The elution gradient established was isocratic 15% B for 5 min, 15–20% B over 5 min, 20–35% B over 10 min, 35–50% B over 10 min, 50–60% B over 5 min, isocratic 60% B for 5 min and re-equilibration of the column to the initial solvent conditions. The flow rate was 0.5 mL min^{-1}. UV detection was performed using 280, 330 and 370 nm as the preferred wavelengths. MS spectra were recorded in the negative ion mode between *m*/*z* 100 and *m*/*z* 1500. Zero grade air served as the nebulizer gas (30 psi) and as the turbo gas (400 °C) for solvent drying (40 psi). Nitrogen served as the curtain (20 psi) and collision gas (medium). Both quadrupoles were set at unit resolution and EMS and EPI analyses were also performed. The EMS parameters were: ion spray voltage 4500 V, DP −50 V, EP −6 V, CE −10 V and cell exit potential (CXP) −3 V, whereas the EPI settings were: DP −50 V, EP −6 V, CE −25 V and CES 0 V. The individual phenolic compounds were tentatively identified by comparing their UV and mass spectra with those reported in the literature, as well as with standards when available. For quantitative analysis, calibration curves were prepared from caffeic acid (for hydroxycinnamoyl derivatives) and kaempferol-3-*O*-glucoside (for flavonoids). The results are expressed in mg per 100 g of dry weight (dw).

2.5. Bioactive properties

2.5.1. Evaluation of the antioxidant activity. DPPH radical-scavenging activity, ABTS radical cation scavenging activity and ferric reducing antioxidant power (FRAP) were determined.[19] Moreovers, Lipid peroxidation inhibion was evaluated using assay thiobarbituric acid reactive substances (TBARS).[20] The results of the determination of the above antioxidant activities are expressed in EC_{50} values, *i.e.* sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay. Regarding the DNA nicking assay,[21] a volume of 5 μL of each extract at a concentration of 2 mg mL^{-1} was added to 2 μL of pGEM®-Tplasmid DNA (0.5 μg per well). The mixtures were then kept for 10 min at room temperature, followed by the addition of 10 μL of Fenton's reagent (3 mM H_2O_2, 50 μM L-ascorbic acid and 80 μM $FeCl_3$). The mixtures were incubated for 5 min at 37 °C and the DNA was then analyzed by 1% (w/v) agarose gel electrophoresis and visualized under UV light.

2.5.2. Antiproliferative properties

Cytotoxicity assays. All the assays were carried out in triplicate and the results are expressed as GI_{50} values in μg mL^{-1} (sample concentration that inhibited 50% of the net cell growth). Ellipticine was used as the positive control in the assays.

Evaluation of cytotoxicity in human tumor cell lines. Four human tumor cell lines were used: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma), as previously described by Dias *et al.*[18]

Evaluation of cytotoxicity in a porcine liver primary cell culture. For the evaluation of the cytotoxicity in non-tumor cells, a cell culture was prepared from a freshly harvested porcine liver (PLP2) according to the procedure established by Dias *et al.*[18]

2.6. Statistical analysis

All the assays were carried out in triplicate. The results are expressed as mean values ± standard deviation (SD), being analysed using a Student's *t*-test, with $\alpha = 0.05$.

3. Results and discussion

3.1. Nutritional value

The results of the nutritional composition (expressed on a fresh weight basis) are shown in Table 1. The young shoots revealed high moisture and dietary fiber content (82.38 and 11.88 g per 100 g, respectively), whereas the other macronutrients were found in lower amounts (0.82, 1.19 and 3.64 g per 100 g for ash, proteins and lipids, respectively).

It is well known that a daily intake of 7 g of plant dietary fiber is considered enough to significantly decrease the menace of cardiovascular and coronary heart diseases.[7] In this sense, the consumption of 100 g of fresh young shoots of *A. spitularis* would cover this daily recommended dose. Thus, wild asparagus can be considered an interesting source of dietary fiber and could be added to other food products to improve fiber intake.

It can be noted that the value of proteins is similar to that reported in cultivated asparagus (*Asparagus officinalis*) young shoots, but less than that reported for wild asparagus (*Asparagus acutifolius*) young shoots. The total lipids amount is higher than the values reported for both the above species.[1] The energy value expressed as kcal per 100 g was 51.57. Comparing our results with those reported for the energy values of selected Mediterranean wild edible plants, *A. stipularis* young shoots showed a higher value than *Anchusa azurea* Mill (24 kcal), *Apium nodiflorum* (L.) Lag. (21 kcal), *Asparagus acutifolius* (40 kcal) and *Beta maritime* L. (31 kcal).[7] The contents of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were evaluated in the young shoots, IDF being the main fraction (11.01 g per 100 g fw; Table 1). Previous studies demonstrated that dietary fiber is able to interfere with enterohepatic bile circulation, decreasing cholesterol and bile acid absorption and causing the depletion of hepatic cholesterol pools.[22]

The micro- and macroelement content, expressed as mg per 100 g fw, is given in Table 1. For microelements, Mn, Zn, Cu and Fe were detected. The Fe content was the highest (1.47 mg

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Table 1 Proximate composition (g per 100 g fw), microelements (mg per 100 g fw), soluble sugars (g per 100 g fw), dietary fiber (g per 100 g fw), organic acids (g per 100 g fw) and energy value (kcal per 100 g fw) of *Asparagus stipularis* Forssk. young shoots

	Content
Proximate composition (g per 100g fw)	
Moisture	82.38 ± 0.35
Crude proteins	3.64 ± 0.11
Total fat	1.19 ± 0.005
Total ash	0.82 ± 0.05
Microelements (mg per 100g fw)	
Fe	1.47 ± 0.07
Cu	0.74 ± 0.03
Mn	0.05 ± 0.002
Zn	0.78 ± 0.02
Macroelements (mg per 100g fw)	
Ca	137.79 ± 8.08
Mg	45.47 ± 1.17
Na	5.00 ± 0.24
K	197.07 ± 4.79
Total soluble sugars (g per 100 g fw)	**0.616 ± 0.002**
Fructose	41.33 ± 0.01
Glucose	125.98 ± 0.02
Sucrose	199.80 ± 0.03
Galactose	32.32 ± 0.30
Arabinose	12.17 ± 0.02
Rhamnose	14.33 ± 0.01
Raffinose	49.32 ± 0.02
Inositol	129.07 ± 0.02
Total dietary fiber (g per 100 g fw)	**11.88 ± 0.19**
Soluble dietary fiber	0.87 ± 0.11
Insoluble dietary fiber	11.01 ± 0.19
Total organic acids (g per 100 g fw)	**1.93 ± 0.01**
Oxalic acid	0.15 ± 0.001
Malic acid	0.19 ± 0.001
Citric acid	1.60 ± 0.006
Energy (kcal per 100 g fw)	51.57 ± 0.49

per 100 g), followed by Zn (0.78 mg per 100 g), Cu (0.74 mg per 100 g) and Mn (0.05 mg per 100 g). The prevalence of iron among the microelement profile is in agreement with the results obtained for *A. stipularis* grown in Turkey,[12] although higher values were reported by those authors comparing with the Tunisian samples analyzed herein. This variation might be explained by the different growing habitats that can affect the nutritional content of plants.[23] Regarding the Cu content, our samples contained three times higher values than green asparagus and two times higher values than white asparagus (*A. officinalis*), as previously reported.[24] By contrast, the Zn content was found to be lower than the one reported by that author for both the other species. On the other hand, the results obtained in our study are in good agreement with the values reported by García-Herrera[25] in wild Spanish asparagus (*A. acutifolius*) in terms of the prevalence of iron among the microelement profile. However, the herein studied plant showed higher contents of Fe (1.47 *vs.* 0.66 mg per 100 g) and Cu (0.74 *vs.* 0.10 mg per 100 g), and lower levels of Mn and Zn than the ones reported by that author.

Regarding the macroelements, Ca, Mg, Na and K were quantified, K and Ca being the major macroelements found in young shoots with values of 197.07 and 137.79 mg per 100 g fw, respectively. In both cases, the obtained results were higher than those reported for *A. acutifolius.*[7] The Na content (5.00 mg per 100 g fw) was much lower than that reported for this wild asparagus (18.5 mg per 100 g fw).[7] Moreover, the oxalic acid/Ca ratio was 1.08 (must be lower than 2.5), which means that the consumption of this wild vegetable reduces the bioavailability of dietary Ca by the formation of an insoluble complex of calcium oxalate.[16]

The concentrations of soluble sugars, namely monosaccharides (mannose, glucose, fructose, galactose, arabinose and rhamnose), sugar alcohols (inositol), disaccharides (sucrose) and oligosaccharides (raffinose) detected in *A. stipularis* young shoots are given in Table 1. The analysis revealed that sucrose was the predominant soluble sugar in the investigated sample. Similar results have been reported by Martins *et al.*[2] who also detected sucrose as the most abundant sugar in wild green asparagus. Moreover, Martins *et al.*[2] reported that, under normal culture conditions, the content of sucrose was generally higher than the glucose and fructose values. Regarding polyols, inositol was detected in appreciable amounts in the herein studied sample. According to Mechri *et al.*,[15] polyols may function as scavengers of activated oxygen species which prevent lipid peroxidation and cell damage.

The results for the determination of fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and the ratios PUFA/SFA and n-6/n-3 are shown in Table 2. Palmitic acid (PA; 16 : 0) was the most abundant saturated acid. PA is one of the most common saturated fatty acids found in animals and plants. The World Health Organization claims that the consumption of PA raises the risk of developing cardiovascular diseases. However, French *et al.*[26] concluded that PA has no hypercholesterolemic effect if the intake of LA (linoleic acid) is above 4.5% of energy. The unsaturated fatty acid percentage was higher (54.67%) than that of SFA (45.43%). Oleic acid (n-9; 18:1) was the main monounsaturated fatty acid (11.62%), while linoleic acid (n-6; C18:2) and α-linolenic acid (n-3; C18:3) were the main PUFA. These results are in agreement with those reported in other wild *Asparagus* species.[3,6] The ratios PUFA/SFA and n-6/n-3 fatty acids were also determined. According to nutritional recommendations, values above 0.45 and lower than 4.0 are highly desirable for PUFA/SFA and n-6/n-3 fatty acid ratios, respectively.[3] Both criteria are met in *A. stipularis* young shoots (Table 2).

3.2. Bioactive compounds

3.2.1. Organic acids. The results for the determination of organic acid contents are presented in Table 1. The average concentration of total organic acids in the analyzed wild asparagus was 1.93 g per 100 g fw. Citric acid was the most abundant (1.58 g per 100 g fw) followed by malic acid (0.18 g per 100 g fw) and oxalic acid (0.15 g per 100 g fw). Similarly, Pereira *et al.*[5] described citric acid as the main organic acid in

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Table 2 Main fatty acid composition (relative percentage of total fatty acids) and tocopherols (mg per 100 g fw) evaluated in *Asparagus stipularis* Forssk. young shoots

Fatty acid	Fatty acid content (relative percentage)
Saturated	
C6:0	0.27 ± 0.075
C12:0	1.45 ± 0.011
C14:0	0.35 ± 0.12
C15:0	0.72 ± 0.06
C16:0	31.09 ± 0.02
C17:0	0.68 ± 0.011
C18:0	4.31 ± 0.011
C20:0	3.13 ± 0.01
C22:0	0.65 ± 0.07
C23:0	0.66 ± 0.02
C24:0	2.12 ± 0.01
Monounsaturated	
C16:1 (n-9)	1.44 ± 0.0
C18:1 (n-9)	11.62 ± 0.007
C20:1 (n-9)	0.75 ± 0.06
Polyunsaturated	
C18:2 (n-6)	31.47 ± 0.009
C18:3 (n-3)	8.66 ± 0.01
C20:2 (n-6)	0.73 ± 0.05
Total SFA	**45.43 ± 0.004**
Total MUFA	**13.80 ± 0.012**
Total PUFA	**40.87 ± 0.002**
PUFA/SFA	**0.89 ± 0.005**
n-6/n-3	**3.71 ± 0.04**
α-Tocopherol	0.06 ± 0.003
γ-Tocopherol	0.07 ± 0.002
Total tocopherols	**0.13 ± 0.001**

Table 3 Volatile compounds (% of total volatile compounds) evaluated in *Asparagus stipularis* Forssk. young shoots

Volatiles	l.r.i.	Content (%)
Hexanal	802	4.1 ± 0.08
Isovaleric acid	836	1.6 ± 0.11
2-Methylbutanoic acid	845	1.7 ± 0.10
p-Xylene	865	1.3 ± 0.11
2,5-Dimethylpyrazine	913	1.6 ± 0.14
Butyrolactone	914	4.6 ± 0.06
(*Z*)-2-Heptenal	961	1.3 ± 0.17
Benzaldehyde	962	3.4 ± 0.09
1-Octen-3-ol	981	3.4 ± 0.011
Hexanoic acid	987	6.4 ± 0.05
Octanal	1002	2.4 ± 0.09
Limonene	1032	11.5 ± 0.01
γ-Terpinene	1063	2.7 ± 0.11
2-Acetylpyrrole	1067	4.6 ± 0.08
n-Undecane	1100	2.5 ± 0.07
Linalool	1101	1.7 ± 0.11
Nonanal	1104	11.7 ± 0.02
Pentylisovalerate	1108	1.5 ± 0.18
(*Z*)-3-Hexenyl isobutyrate	1143	2.3 ± 0.16
2-Ethylhexyl acetate	1155	5.6 ± 0.06
8-Methylnonanal	1172	1.6 ± 0.15
α-Terpineol	1191	1.8 ± 0.14
n-Dodecane	1200	2.7 ± 0.06
Decanal	1206	4.7 ± 0.06
(*E*)-2-Decenal	1263	1.6 ± 0.27
n-Tridecane	1300	1.5 ± 0.20
n-Tetradecane	1400	3.5 ± 0.10
β-Caryophyllene	1419	1.4 ± 0.17
(*E*)-Geranylacetone	1455	1.6 ± 0.14
n-Pentadecane	1500	2.2 ± 0.17
Monoterpene hydrocarbons	—	14 ± 0.03
Oxygenated monoterpenes	—	3.30 ± 0.10
Sesquiterpene hydrocarbons	—	1.40 ± 0.14
Apocarotenes	—	1.60 ± 0.06
Nitrogen derivatives	—	6.00 ± 0.04
Non-terpene derivatives	—	68.1 ± 0.002
Total identified		98.6

l.r.i.: Linear retention index.

the young shoots of other wild asparagus samples (*Asparagus acutifolius*).

3.2.2. Tocopherols. In the analysed sample, α-tocopherol (0.06 mg per 100 g fw) and γ-tocopherol (0.07 mg per 100 g fw) were determined (Table 2); the δ- and β-isoforms were not detected, opposite to what was reported in other wild asparagus species (*A. acutifolius*) by Morales *et al.*[4] Nevertheless, the latter authors also found that α- and γ-tocopherol were the main ones responsible for total vitamin E activity in their asparagus samples. Expressing the results on a dry weight basis, the total tocopherol content (2.1 mg per 100 g) of cultivated *A. officinalis* and wild *A. acutifolius* (2.78 mg per 100 g) were higher than the one obtained herein (0.162 mg per 100 g).[4,27]

3.2.3. Volatile compounds. Solid-phase micro-extraction coupled with GC-MS was used for the characterization of the volatile compounds in *A. stipularis* young shoots (Table 3). Thirty compounds were identified, accounting for 98.6% of the total emitted volatiles. They included alcohols, esters, aldehydes, hydrocarbons, terpenoids, apocarotenes and others, aldehydes and hydrocarbons being the main chemical classes. Aldehydes were also described as the major volatile compounds for three genotypes of *A. officinalis* reported by Sun *et al.*[28] Among aldehydes, hexanal, heptenal, octanal, benzaldehyde, nonanal and decanal were detected. The same compounds were also reported by Chin *et al.*[28] in the volatile composition of green asparagus juice. Nonanal, the major aldehyde (11.7%) in the studied samples, has been used for different purposes, such as the improvement and monitoring of oral breath odour and the diagnosis of some human illnesses.[29]

Among terpenoids, limonene showed the highest percentage in *A. stipularis* young shoots. It has been employed to prevent gastric diseases[30] and it is also known for its antiproliferative effects.[31] As for alcohols, 1-octen-3-ol was detected. This alcohol is also known as mushroom alcohol because it is the major component of the volatiles in cultivated mushroom, and it is the dominant contributor to the characteristic mushroom flavour.[27] The analysis of volatiles can be a useful tool to provide an objective guide for the analysis and improvement of the flavour characteristics of wild asparagus, as well as other crops.

3.2.4. Phenolic compounds. The phenolic profile of *A. stipularis* young shoots was characterized by the presence of flavonol and hydroxycinnamoyl derivatives. Thirteen main

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Table 4 Retention time (Rt), wavelengths of maximum absorption in the UV-vis region, mass spectrometric data, tentative identification and quantification of individual phenolic compounds in wild *Asparagus* young shoots (mg per 100 g of dw)

Peak	Rt (min)	UV-vis max (nm)	[M − H] (*m*/*z*)	MS^2 (*m*/*z*)	Compound	Infusion	Hydroalcoholic extract	*p*-Value
1	7.4	331	355	193, 174, 134	Feruloylhexoside	1.36 ± 0.05	1.47 ± 0.07	>0.05
2	11.0	254, 349	755	300, 271	Quercetin-*O*-dideoxyhexosylhexoside	1.08 ± 0.17	1.22 ± 0.17	>0.05
3	13.9	265, 347	739	327, 284, 255	Kaempferol-*O*-dideoxyhexosylhexoside	5.01± 0.17	4.87 ± 0.40	>0.05
4	14.3	254, 353	769	314, 299, 271	Isorhamnetin-O-dideoxyhexosylhexoside	3.02 ± 0.11	2.66 ± 0.33	>0.05
5	15.0	324	267	193, 175, 160, 134	Feruloyl glycerol	1.20 ± 0.04	1.10 ± 0.16	>0.05
6	15.4	266, 350	609	301	Quercetin-3-*O*-rutinoside	1.02 ± 0.08	1.10 ± 0.06	>0.05
7	16.3	320	853		Unknown hydroxycinnamoyl derivative	1.30 ± 0.03	1.18 ± 0.05	>0.05
8	18.1	254, 353	623	315, 300, 271	Isorhamnetin-3-*O*-rutinoside	0.88 ± 0.01	0.99 ± 0.27	>0.05
9	20.1	324	459	241, 139	Unknown hydroxycinnamoyl derivative	1.02 ± 0.08	0.98 ± 0.11	>0.05
10	29.3	315	413	193, 163, 145, 134	Coumaroylferuloyl glycerol I	3.03 ± 0.12	3.86 ± 0.43	<0.05
11	29.8	325	443	193, 175, 161, 135	Diferuloyl glycerol I	5.38 ± 0.01	7.07 ± 0.80	<0.05
12	30.1	313	413	193, 161, 145, 134	Coumaroylferuloyl glycerol II	0.22 ± 0.03	1.05 ± 0.12	<0.05
13	30.6	324	443	193, 175, 160, 134	Diferuloyl glycerol II	0.55 ± 0.01[a]	1.95 ± 0.10[b]	<0.05
					Total flavonoids	11.03 ± 0.42	10.86 ± 0.65	>0.05
					Total phenolic acid derivatives	14.10 ± 0.14	18.69 ± 1.05	<0.05
					Total phenolic compounds	25.13 ± 0.55	29.56 ± 1.52	<0.05

$p < 0.05$ means significant differences.

compounds were detected, and the peak characteristics (UV absorption and mass spectra), tentative identities and quantitative results are presented in Table 4. Five peaks were associated with flavonol glycosides according to their characteristic UV spectra that were identified as quercetin, kaempferol, and isorhamnetin derivatives based on their mass spectral data. The types of sugar substituents were deduced from the mass difference between the *m*/*z* values of the pseudomolecular ions and the aglycones, *i.e.*, 454 Da (peaks 2, 3 and 4) and 308 Da (peaks 6 and 8), coherent with combinations of deoxyhexosyl (146 Da) and hexosyl moieties (162 Da). In all cases, sugar substituents were assumed to be in the form of di- or trisaccharides; otherwise, fragments corresponding to the alternative loss of the distinct sugars should have been observed. Thus, compounds 6 and 8 would contain one hexosyl and one deoxyhexosyl residue, while compounds 2, 3 and 4 have one hexosyl and two deoxyhexosyls. Peaks 6 and 8 were tentatively assigned to quercetin-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside, owing to their previous identification in different species of *Asparagus*.[8,32] Based on this assumption, peaks 2, 3 and 4 might be supposed to correspond to the rhamnosyl-rutinosides of quercetin, kaempferol and isorhamnetin, respectively. However, since no definite conclusion about the nature and location of the sugars can be obtained from the mass spectral data, they were just assigned to *O*-dideoxyhexosyl-hexosides. Two compounds possessing $[m - H]^-$ ions at *m*/*z* 755 and 769, as here obtained for peaks 2 and 4, were reported in green asparagus by Fuentes-Alventosa *et al.*,[33] although they were just designed as quercetin and isorhamnetin triglycosides.

The remaining eight peaks were associated with hydroxycinnamoyl derivatives, according to their UV spectra showing characteristic shapes with maximum wavelengths of absorption at 320–330 nm. Among them, peaks 1, 5 and 10–13 were assigned to different feruloyl derivatives based on their mass features as previously described in *A. officinalis*.[33] Compounds 10 and 12 showed the same pseudomolecular ion ($[m - H]^-$ at *m*/*z* 413) and this similarly happened with compounds 11 and 13 ($[m - H]^-$ at *m*/*z* 443), which can be explained by the substitution of the feruloyl/coumaroyl residues on any of the three hydroxyl groups of glycerol. Actually, the presence of 1,2-*O*-diferuloyl glycerol and 1,3-*O*-diferuloyl glycerol was reported in *A. officinalis*,[33,34] which could correspond to the peaks 11 and 13 detected herein. No conclusions on the identity of peaks 7 and 9 could be obtained, which remained as unknown hydroxycinnamoyl derivatives.

3.3. Bioactive properties

3.3.1. Antioxidant activity. The antioxidant activity of *A. stipularis* young shoot extracts was evaluated using four different methods: DPPH and ABTS radical scavenging activity, ferric reducing power assay and TBARS determination in brain homogenates. As it can be seen in Table 5, for all the antioxidant activity assays, the hydroalcoholic extract gave the lowest EC_{50} value. ABTS, DPPH and ferric reducing power assays were applied to evaluate the total antioxidant capacity, obtaining EC_{50} values of 3.86, 2.20 and 1.19 mg mL^{-1} for the hydroalcoholic extract, respectively. As for the infusion, ABTS, DPPH and ferric reducing power assays gave values of 7.41, 6.16 and 3.70 mg mL^{-1}, respectively. The TBARS assay was used for the evaluation of lipid peroxidation. The values of 3.19 and 0.86 mg ml^{-1} were obtained for infusion and hydroalcoholic extracts, respectively. The higher antioxidant activity of the hydroalcoholic extract was probably related to the higher content of total phenolic compounds found in this extract, compared to the infusion (Table 5). According to Fuentes-Alventosa *et al.*,[32] the correlation between phenolic compounds and the antioxidant activity of asparagus has been previously reported. Comparing the results with other wild Mediterranean species, the *A. stipularis* hydroalcoholic extract has greater antioxidant activity measured by DPPH and redu-

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Table 5 Antioxidant (EC_{50} values, mg mL^{-1}) and cytotoxic activities (GI_{50} values μg mL^{-1}) of *A. stipularis* young shoots

	Infusion	Hydroalcoholic extract	*p*-Value
DPPH scavenging activity	6.16 ± 0.003^{b}	2.206 ± 0.14^{a}	<0.05
ABTS scavenging activity	7.41 ± 0.051^{b}	3.86 ± 0.07^{a}	<0.05
Reducing power assay	3.70 ± 0.019^{b}	1.19 ± 0.023^{a}	<0.05
TBARS inhibition assay	3.19 ± 0.17^{b}	0.86 ± 0.011^{a}	>0.05
MCF-7 (breast carcinoma)	>400	298.63 ± 4.51	—
NCI-H460 (non-small cell lung cancer)	>400	244.26 ± 8.82	—
HeLa (cervical carcinoma)	>400	208.24 ± 6.02	—
HepG2 (hepatocellular carcinoma)	>400	200.77 ± 3.51	—
PLP2	>400	>400	_

EC_{50}: Extract concentration corresponds to 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay. The GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in human tumour cell lines or in liver primary culture PLP2. $p < 0.05$ means significant differences.

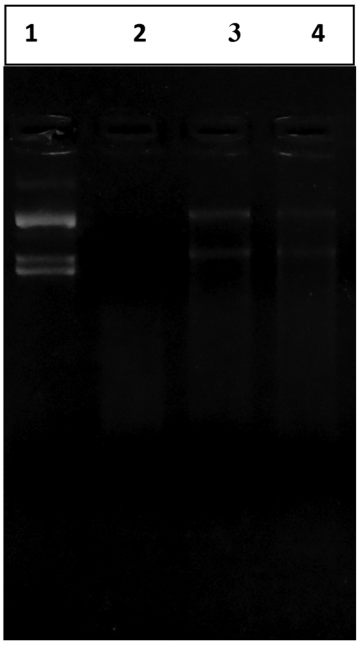

Fig. 1 Inhibitory effects of *A. stipularis* young shoot extracts on DNA nicking caused by hydroxyl radicals. Lane 1: native pGEM®-Tplasmid DNA; lane 2: Fenton's reagent + DNA; lane 3: Fenton's reagent + DNA + infusion (2 mg ml^{-1}); lane 4: Fenton's reagent + DNA + hydroalcoholic extract (2 mg mL^{-1}).

cing power assays than the Spanish samples of *Asparagus acutifolius*, *Bryonia dioica*, *Scolymus hispanicus* L., *Silybum marianum* (L.) and *Tamus communis* L. previously analyzed.[4] As for infusion, the DPPH and FRAP values of *A. stipularis* were higher than those reported for the Portuguese *Foeniculum vulgare* Mill. and Spanish *Scolymus hispanicus* L. samples.[2,4]

Regarding the *DNA nicking assay*, the effect of *A. stipularis* extracts was evaluated qualitatively on supercoiled pGEM®-Tplasmid DNA. According to Abbas *et al.*,[35] hydroxyl radicals generated by the Fenton reaction are responsible for oxidatively induced breaks in the DNA strands. The obtained electropherograms of the current assay are shown in Fig. 1. The direction of migration is from the lower to upper part of the electropherograms. The faster moving band corresponds to the supercoiled circular DNA and the slower moving band represents the open circular DNA following treatment with Fenton's reagent. The control (lane 2) showed the absence of specific band in the treated DNA, which indicates DNA damage. The addition of the asparagus young shoot infusion (lane 3) and hydroalcoholic extract (lane 4) to the reaction mixture decreased the DNA strand scission and retained the supercoiled form, thus effectively protecting the DNA. To the best of our knowledge, this is the first report on the DNA protecting ability of *A. stipularis* extracts.

3.3.2. Antiproliferative activity. The antiproliferative properties of the asparagus young shoot extracts were evaluated *in vitro* by the estimation of cell growth effects on four human tumour cell lines (MCF-7, HCT-15, HeLa and HepG2). The results are shown in Table 5. All the cell lines were inhibited in a dose-dependent manner by the hydroalcoholic extract. The lowest GI_{50} values were obtained for HepG2 and HeLa (200.77 and 208.24 μg mL^{-1}, respectively). A possible explanation for this observation could be the presence of steroidal saponins in the extract, which have been reported to have antitumor activity.[35] Asparanin A isolated from *A. officinalis* has been found to induce cell cycle arrest in human hepatocellular carcinoma HepG2 cells.[36] Anti-cancer properties against HL-60 (human promyelocytic leukemia) and Hep G2 cells were also demonstrated for ursolic acid isolated from *A. officinalis* young shoots.[37] The infusion extract had no antitumor effects at the maximal concentration used (400 μg mL^{-1}). Despite the observed cytotoxic activity on human tumour cell lines, none of the *A. stipularis* preparations showed hepatotoxicity in the porcine liver primary cell culture (non-tumour cells; PLP2).

This study is a contribution to the overall knowledge on the phytochemistry and biopotential of *A. stipularis* young shoots. According to the obtained results, *A. stipularis* young shoots are a good source of essential and non-essential nutrients. The hydroalcoholic extract of the young shoots exhibited appreciable antioxidant and antiproliferative activities compared to the aqueous extract. Both extracts were found to be non-hepatotoxic against PLP2 cells up to the maximal tested concentration (400 μg mL^{-1}). The phenolic profile of *A. stipularis* young shoots was characterized by the presence of flavonol and hydroxycinnamoyl derivatives as the main compounds.

In conclusion, *A. stipularis* might be an interesting candidate to improve and recover wild plant consumption, including it young shoots as part of the Mediterranean diet in the so-called modern diets rich in antioxidant and biofunctional foods and food product, as well as for the development of new antioxidant and cancer chemopreventive dietary supplements or nutraceuticals.

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Conflicts of interest

The authors declare that have no conflicts of interest.

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