Wild edible Swiss chard leaves (*Beta vulgaris* L. var. *cicla*): Nutritional, phytochemical composition and biological activities

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Abstract

Nutritional, soluble carbohydrates and aroma volatile profiles of Tunisian wild Swiss chard leaves (*Beta vulgaris* L. var. cicla) have been characterized. The chemical composition of an ethanol chard leaves extract, as well as its *in vitro* antioxidant, α-amylase and α-glucosidase inhibition activities were carefully evaluated. The results of the proximate composition showed that total carbohydrate fraction, mainly as dietary fiber were the major macronutrient (2.43 g/100g fw), being the insoluble dietary fiber the predominant fraction (2.30 g/100g). In addition, leaves of *Beta vulgaris* L. were especially rich in Mg, Fe and Ca (4.54, 2.94 and 2.28 mg/100g fw) and very poor in Na (0.09 mg/100g fw). Volatile profile revealed that non-terpene derivatives and sesquiterpene hydrocarbons were the essential classes of volatiles in the chard leaves. Myricitrin, p-cumaric acid and rosmarinic acid were characterized. Moreover, the ethanol extract of wild Swiss chard leaves revealed significant antioxidant capacity. Furthermore, a good enzyme inhibitory effects on α-glucosidase and α-amylase activities were observed. These findings highlighted the potential health benefits of wild Swiss Chard as a source of nutritional and bioactive compounds.

Keywords: *Beta vulgaris*; halophyte plant; wild plant; nutritional profile; aroma volatiles; antioxidant properties; enzyme inhibitory activity.

1. Introduction

Halophytic plants have extensive tradition of consumption in over the world which due to their organoleptic properties (flavor, smell, appearance, among others) (Ventura & Sagi, 2013). Moreover, it has been reported that halophytes provide nutritional profiles suitable for human consumption (Barreira et al., 2017).

Wild vegetables in the Mediterranean diet have been proved to be protective against miscellaneous chronic diseases, such as cancer, cardiovascular diseases, brain disorders, immune dysfunctions, among others (Sánchez-Mata & Tardío, 2016; Septembre-Malaterre, Remize, & Poucheret, 2017). In this context, considerable interest has been focused during the last years on searching nutritional and phytochemical enhancement of wild vegetables in order to reduce micronutrient malnutrition. Since, the malnutrition, especially iron deficiency, is a serious and widespread public health problem due to the Food and Agriculture Organization (FAO) recommendations (Thompson, 2007). The causes of this disease are multiple but above all nutritional characterized by a low contribution of hematopoietic factors (iron, vitamin B_{12}, folate). The low bioavailability of iron in our daily diet is the main impediment to the coverage of people's iron needs. Thus, the valorization of plant varieties is one of the strategies to fight against micronutrient deficiencies.

The wild Swiss chard (*Beta vulgaris* L. var. cicla), is a glycophytic belonging to the Chenopodiaceae family, that is distributed all over the world and employed as a leafy green vegetable for its year round availability and low cost (Ustundag et al., 2016). There are a few reports about Swiss chard resistance to salt stress, which demonstrate that Swiss chard showed marked osmotic adjustment under salt stress (Ghoulam, Foursy, & Fares, 2002). Thus, the Swiss chard could be considering as halophyte plant that play a considerable role in human Mediterranean diet and it has a widespread use in many traditional dishes because of its nutritional values and minerals presence (Gao, Han, & Xiao, 2009). The stalks are

commonly chopped and cooked like celery, while the leaves might be accustomed in salads or cooked like spinach. Traditionally, chard has been employed for its beneficial health effects as a folk remedy for kidney and liver diseases, for stimulation of the immune and hematopoietic systems, and as a distinctive diet in the cancer treatment (Kanner, Harel, & Granit, 2001). The phytochemical screening of chard revealed the existence of some fatty acids (stearic, palmitic, linoleic oleic, and linolenic acids), phospholipids, glycolipids, polysaccharides, ascorbic acid, folic acid, pectins, saponins, flavonoids (apigenin), phenolic acids, and betalains (Gao et al., 2009). The significance of these bioactive molecules from chard extracts has been addressed and their antioxidant, anti-acetylcholinesterase, anti-diabetic, anti-inflammatory, antitumor and hepatoprotective effects have been demonstrated (Gezginci-Oktayoglu et al., 2014; Oztay et al., 2015). Besides, they exhibit mineralizing, antiseptic and choleretic activities as well as they contribute to the reinforcement of the gastric mucosa. Moreover, the stalks of this vegetable nutritionally were an interesting source of dietary fibers, vitamins A, C E, K, B, calcium, iron, phosphorus, zinc, magnesium, potassium, copper and manganese (Gennari et al., 2011).

It´s well establish that wild plants, mainly halophytes plants, present a higher amount of secondary metabolites (e.g. phenolic compounds synthetized during stress conditions, as UV or salt resistant stress) with relevant biological activities (Barros, Morales, Carvalho & Ferreira, 2016). Thus, the increasing interest in nutritional and pharmacological potential of chard allowed us to examine the chemical wild chard leaves content, which is less known in comparison with copious literature reported on its stalks and seeds.

Therefore, in the present study, chemical composition and full nutritional value of wild Tunisian Swiss chard leaves were evaluated in order to highlight their pivotal role in human diet, comparing with its cultivated relatives. The leaves volatile composition, never reported before, was also screened. Furthermore, the biological (antioxidant, α-amylase and α-

glucosidase inhibition) activities of ethanol extract, prepared from Swiss chard leaves, were assessed.

2. Material and Methods

2.1. Plant Material

Leaves of wild Swiss chard (*Beta vulgaris* L. var. cicla) were collected in Monastir (Tunisian Sahel; coordinates: Lat. 35°73′N; Long.10°76′E), Tunisia in March 2017. The plant material was collected and identified by Pr. Fethia Harzallah Skhiri (High Institute of Biotechnology of Monastir, Tunisia). Then, the leaves were freeze-dried, cut into little pieces and ground using a blender to obtain fine powder and stored in dark bags at 4 °C until analysis.

2.2. Chemicals and reagents

The following chemicals reagents were used in this study: $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt ($ABTS^{+\bullet}$), linoleic acid, trichloroacetic acid (TCA), gallic acid and Folin-Ciocalteu's reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA). Micro (Fe, Cu, Mn and Zn) and macroelement (Ca, Mg, Na and K) standards (>99% purity), as well as $LaCl_2$ and CsCl (>99% purity) were purchased from Merck (Darmstadt, Germany). Solvents were commercially obtained (Sigma Aldrich) at the highest commercial quality and used without further purification. All other reagents used were of analytical grade.

2.3. Nutritional composition

2.3.1. Proximate analysis

The powdered plant material was analyzed for moisture, fat, proteins, ash and carbohydrates based on the AOAC methods (AOAC, 1995). The fat was evaluated by extracting in a Soxhlet a known weight of powdered material with petroleum ether; the protein content (N×6.25) was

estimated by the macro-Kjeldahl technique; the ash content was estimated via incineration at 600 ± 15°C. The total carbohydrates were obtained by difference.

2.3.2. Soluble sugars determination

Chard soluble carbohydrates were extracted based on the method described by Bartolozzi et al (1997) (Bartolozzi, Bertazza, Bassi, & Cristoferi, 1997). The extract was dried and transformed into trimethylsilyl ethers by treatment with pyridine, hexamethyldisilazane and trimethylchlorosilane. Soluble sugars were analyzed using a Hewlett Packard 5890 series II gas chromatograph equipped with a flame ionization detector (FID) and a HP-5MS column (30 m x 0.25 mm). Identification of individual soluble sugars was achieved by mean of relative retention times, in comparison to that of standards. These were compared to those already identified by gas chromatography mass spectrometry. The soluble sugars contents were expressed as g per 100 g of fresh weight (fw).

2.3.3. Soluble and insoluble dietary fiber assay

Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) was estimated according to AOAC enzymatic-gravimetric method (AOAC, 2005). The sum of both fractions, SDF and IDF, correspond to total dietary fiber (TDF) content.

2.3.4. Fatty acids profile

The oil fatty acids were transformed into their methyl esters using a boron trifluoride methanol complex (14% w/v). The blend was maintained at 100°C for 1h. The reaction was stopped with 0.5 mL of deionized water. Then, the extracted fatty acid methyl esters (FAME) were dissolved in pure hexane for GC analyses. The individual FAMEs were separated and quantified by gas chromatography using a model 5890 Series II instrument (Hewlett-Packard, Palo-Alto, Ca, USA) equipped with a flame ionization detector, and a fused silica capillary column DB23 capillary column (60 m length, 0.32 mm i.d., and 0.25 μm film thickness; HP-Agilent Technologies, Wilmington). Oven temperature was set at 130°C, increased to 170°C

at 6.5 °C/min, then augmented more again to 215°C at 2.8 °C/min and was held there for 12 min. Lastly, it was increased to 230 °C at 40°C/min and maintained for 20 min. The injector and detector temperatures were set at 270 °C and 280 °C, respectively. Nitrogen was used as the carrier gas at 1 ml/min and the split ratio was set at 1:5. The FAMEs were identified by comparing their retention times with respect to pure standard FAMEs purchased from Sigma and analyzed under the same conditions (de Britto Policarpi et al., 2017) .

Chard leaves FAMEs were quantified according to their percentage area, obtained by integration of the peaks. Data were expressed as a percentage of individual fatty acids in the lipid fraction.

2.3.5. Mineral analysis (macro and microelements)

Mineral analysis was performed following the procedure describe by Dias et al., 2016. Briefly, 500 mg of sample was incinerated in a microwave oven (Muffle Furnace mLs1200, Thermo Scientific, Madrid, Spain) for 24 h at 550°C, and ashes were gravimetrically quantified. The incineration residue was extracted with HCl (50%, *v/v*) and HNO_3 (50%, *v/v*) and made up to an appropriate volume with distilled water, where Fe, Cu, Mn and Zn were directly measured. An additional 1/10 (*v/v*) dilution of the sample fraction and standards was performed to avoid interferences between diverse elements in the atomic absorption spectroscopy: 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% Cs Cl. For all the atomic absorption spectroscopy (AAS) analysis, an Analyst 200 Perkin Elmer (Perkin Elmer, Waltham, MA, USA) was used, comparing absorbance responses with N99.9% purity analytical standard solutions for AAS made with Fe $(NO_3)_3$, Cu $(NO_3)_2$, Mn $(NO_3)_2$, Zn $(NO_3)_2$, Na Cl, K Cl, Ca CO_3 and Mg band, supplied by Merck (Darmstadt, Germany) and Panreac Química (Barcelona, Spain).

2.4. Determination of volatile compounds

The solid phase microextraction (SPME) analyses were carried out as earlier described by El Arem et al., with minor modifications (El Arem et al., 2012). Supelco (Bellefonte, PA) SPME devices coated with poly-dimethylsiloxane (PDMS, 100 μm) were used to sample the head-space of dry plant material inserted into a 5 mL vial and allowed to equilibrate for 30 min. After the equilibration time, the fiber was exposed to the headspace for 50 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transported to the injection port of the GC–MS system. All the SPME sampling and desorption conditions were identical for all the samples. Moreover, blanks were performed before each first SPME extraction and randomly repeated during each series. Quantitative comparisons of relative peaks areas were carried out between the same chemicals in the different samples.

GC-EIMS analyses were executed with a Varian (Palo Alto, CA) CP3800 gas chromatograph equipped with a DB-5 capillary column (30 m - 0.25 mm - 0.25 μm; Agilent) and a Varian Saturn 2000 ion trap mass detector. The analytical settings were as follows: injector and transfer line temperatures were 250 and 240°C, respectively; oven temperature was programmed from 60 to 240°C at 3 °C/min; carrier gas was helium at 1 mL/min; splitless injection. Constituents identification was based mainly on a comparison of the retention times with those of authentic samples, comparing their linear retention indices and on computer matching against the commercial (NIST 2014 and Adams 2007) and homemade library of mass spectra, and MS literature data. Furthermore, the molecular weights of all the elements identified were established by GC–CIMS, using methanol as ionising gas.

2.5. Bioactive compounds

2.5.1. Pigment composition

Pigments extraction was performed following a technique earlier described by Nagata & Yamashita (1992). Briefly, 500 mg of dried powder was carefully shaken for 1 min with 10 mL of an acetone-hexane mixture (4:6) and filtered. The absorbance (A) of the filtrate was read at 453, 505, 645 and 663 nm. Content of β-carotene and lycopene were expressed using the equations as below:

(1) β-carotene (mg/100 mL) = 0.216×A663 − 1.220×A645 − 0.304×A505 + 0.452×A453

(2) Lycopene (mg/100 mL) = −0.0458×A663 + 0.204×A645 − 0.304×A505 + 0.452×A453

and expressed as mg/100 g of fresh weight (fw).

Chlorophyll content (Ca and Cb) and that of the total chlorophylls (CTC) were designed as mg/100g fresh weight (fw).

2.5.2. Phenolic compounds

2.5.2.1. Preparation of ethanol extract of wild Swiss chard leaves

First a wild Swiss chard ethanol extract was obtained. The sample was extracted by stirring with 40mL of ethanol at 25 °C for 1 h and filtered via Whatman N° 4 filter paper. The residue was then extracted with an additional 40 mL portion of ethanol. The combined ethanol extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland), re-dissolved in ethanol to obtain a concentration of 5 mg/mL, and reserved at 4 °C until use.

2.5.2.2. Total phenolics content

Total phenolics content was estimated based on Stojovic method (Stojković et al., 2014). In fact, an aliquot of the chard ethanol extract (1 mL) was mixed with Folin–Ciocalteu reagent (5 mL, previously diluted with water 1:10 (v/v)) and sodium carbonate (75 g/L, 4 mL). The tubes of appropriate dilutions of the ethanol extract were homogenized for 15 s and allowed to stand for 30 min at 40 °C for color development. Absorbance was then read at 765 nm.

Gallic acid was employed as standard for the calibration curve, and results were expressed as mg of gallic acid equivalents (GAE) per g of chard extract.

2.5.2.3. Total flavonoids content

The total flavonoids content was determined according to (Zhishen, Mengcheng, & Jianming, 1999). An aliquot (0.5 mL) of the chard solution was mixed with distilled water (2 mL) and subsequently with a $NaNO_2$ solution (5%, 0.15 mL). After 6 min, an $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand further 6 min. Afterwards, a NaOH solution (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to adjust the final volume to 5 mL. Then, the blend was properly mixed and allowed to stand for 15 min. The intensity of the pink color was measured at 510 nm. (+)-Catechin was employed to prepare the standard curve and outcomes were expressed as mg of (+)-catechin equivalents (CE) per g of chard extract.

2.5.2.4. *o-Diphenol*s content

The *o-diphenols* content in chard extract was measured according to Mekni et al. (2013) (Mekni, Azez, Tekaya, Mechri, & Hammami, 2013). To 100 µl of sample, 1 mL of a solution of HCl (0.5 N), 1 mL of a solution of a mixture of $NaNO_2$ (10 g) and $NaMoO_4 \cdot 2H_2O$ (10 g) in 100 mL H_2O, and finally 1 mL of a solution of NaOH (1 N) were added. After 30 min, the amount of *o*-diphenols was calculated by measuring the absorbance at 500 nm. The *o*-diphenols were expressed as mg hydroxytyrosol equivalents per g of chard extract.

2.5.2.5. Condensed tannins content

The condensed tannins were determined based on the (de Britto Policarpi et al., 2017) method. An aliquot (50 µL) of chard extract or standard solution was mixed with 1.5 mL of a 4% vanillin methanol solution, and then 750 µL of HCl were added. The solution was incubated for 20 min and the absorbance against a blank was determined at 500 nm. Data were expressed as mg (+)-catechin equivalents (CE) g/extract.

2.5.2.6. Total flavonols

The flavonols content was assessed based on the methods proposed by Miliauskas et al (2004) (Miliauskas, Venskutonis, & Van Beek, 2004). In a tube of eppendorff, the ethanol extract was diluted 1/10 with 10% ethanol. Therefore, to the obtained solution 250 µL of a solution of 0.1% HCl in 95% ethanol and 1 mL of 2% HCl were appended. The solution was blended and allowed to sit for around 15 min before reading its absorbance at 360 nm. The flavonols content was estimated as mg rutin equivalents g of extract.

2.5.2.7. Individual phenolic compounds identification

The identification of phenolic compounds was done using HPLC system (consisting of a vacuum degasser, an autosampler, and a binary pump with a maximum pressure of 400 bar; Agilent 1260, Agilent technologies, Germany) equipped with a reserved phase C18 analytical column of *4.6 x 100 mm and 3.5 µm* particle size (Zorbax Eclipse XDB C18). The DAD detector was set to a scanning range of 200-400 nm. Column temperature was maintained at 25°C. The injected sample volume was 2 µl and the flow rate of mobile phase was 0.4 mL/min. Mobile phase B was milli-Q water consisted of 0.1% formic acid and mobile phase A was Methanol. The optimized gradient elution was illustrated as follows: 0-5 min; 10-20 % A; 5-10 min; 20-30 % A; 10-15 min; 30-50 % A; 15-20 min; 50-70 % A; 20-25 min; 70-90 % A; 25-30 min; 90-50 % A; 30-35 min, return to initial conditions.

The phenolic compounds which were identified by comparison of their retention time and spectra with those obtained from the corresponding standards were as follows: p-coumaric acid, myricitrin acid, and rosmarinic acid.

For the quantitative analysis, a calibration curve was obtained by plotting the peak area against different concentrations for each identified compound using the available standards at 340nm: p-coumaric acid (y = 32.266x + 17.439); myricitrin acid (y = 6.7915x+ 35.235) and rosmarinic acid (y = 8.4942x + 71.265) The obtained curves showed a good linearity (with an

average of R^2 = 0.998). The amount of each compound was expressed as milligram per gram of residue and the final concentration of compounds present in the samples was determined as average content after three consecutive injections.

2.6. Determination of biological activities

In our study, the ethanolic extract, prepared from Wild Swiss Chard leaves, was evaluated via various biological activities.

2.6.1. Antioxidant activity tests

2.6.1.1. DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl radical scavenging) assay

DPPH$^{\bullet}$ free radical scavenging abilities of the chard extract was performed via a method qualified and described by (Dias et al., 2014) with slight modifications. About 1 mL of the ethanol extract at several different concentrations were mixed with 2 mL of a fresh prepared DPPH solution (0.2 mM in methanol). The blend was incubated at 25 °C for 30min, and the absorbance was read at 517 nm with a UV–vis spectrophotometer (Perkin Elmer Lambda 40 UV/VIS Spectrophotometer). Radical scavenging properties were assessed as a percentage of DPPH radical elimination using the equation as below:

$$(3)\ \text{Percent Inhibition (\%)} = \left(1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100$$

2.6.1.2. ABTS$^{+\bullet}$ radical scavenging ability assay

The ABTS$^{+\bullet}$ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) antioxidant activity test was realized based on the earlier reported method by (Moreno-Montoro, Olalla-Herrera, Gimenez-Martinez, Navarro-Alarcon, & Rufián-Henares, 2015). The procedure involved, mixing of ABTS diammonium salt (0.35 mL at 7.4 mmol/L) with potassium persulfate (0.35 mL at 2.6 mmol/L) to obtain ABTS+• radical. To allow complete radical formation, the blend was preserved for 15 h in a dark room at room temperature in a vial with aluminum foil wrapped around. To get an absorbance reading of 0.70 ± 0.02 at 734 nm, the radical solution was diluted with 95% ethanol (about 1:40, v/v). The scavenging ability was evaluated by

adding 2mL of $ABTS^{+\bullet}$ solution 0.2 mL of polysaccharide fractions (95% ethanol was employed as negative control). Absorbance was read at 734 nm 20 min after the initial mixing. The scavenging capacity was calculated as follows:

$$(4)\ \text{Percent Inhibition (\%)} = \left(1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100$$

2.6.1.3. Reducing power assay

The ferric-reducing power was determined following to the method described elsewhere (Dias et al., 2014). Briefly, 250 µL of chard sample extract in distilled water solution at different concentrations (0.5–4 mg/mL) was mixed with 625 µL of sodium phosphate buffer (0.2 M, pH 6.6) and 625 µL of $K_3Fe(CN)_6$ (1%, w/v). The solutions were incubated at 50 °C for 20 minutes. After cooling, 625 µL of TCA (10%, w/v) was added to the mixture to end the reaction. Then, the fractions were centrifuged at 2000 *g* for 10 min. Afterwards, 625 µL were mixed with 625 µL of water and 125 µL of a 1% (w/v) $FeCl_3$ solution, and let stand for 10 min. Absorbance was read at 700 nm against a blank.

2.6.1.4. β-Carotene bleaching inhibition assay

Inhibition of β-carotene bleaching was evaluated through the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which is measured by the formula: β-carotene absorbance after 2 h of assay/initial absorbance) × 100 (Dias et al., 2014).

2.6.1.5. Linoleic acid peroxidation test

In this assay, antioxidant capacity is determined via measuring the thiobarbituric acid-reacting entities (TBARS) may arise from linoleic acid peroxidation (Dias et al., 2014). The reaction blend, which contained 500 µL linoleic acid (20 mM), 500 µL Tris HCl (100 mM, pH 7.5), 100 µL $FeSO_4.7H_2O$ (4 mM) and different concentrations of each processed sample. The peroxidation of linoleic acid was induced by addition of 100 µL of ascorbic acid (2 mM) let to incubate for half an hour at a 37°C and achieved through the addition of 2 mL of TCA (10%). In addition, to 1 mL of the blend, 1 mL of TBA (1%) was added, followed by heating at 95°C

for 10 min in a water bath. Subsequently, the blends were centrifuged at 3500 *g* for 10 min. The TBARS absorbance in the liquid supernatant was estimated at 532 nm according to the equation:

$$(5)\ \text{Linoleic acid peroxidation inhibition (\%)} = [\frac{(Ac-As)}{(Ac-An)}] \times 100$$

Ac = Absorbance of control (without extract)

As = Absorbance of extract

An = Absorbance of blank (without extract and $FeSO_4.7H_2O$).

2.6.1.6.DNA nicking test

The DNA nicking test was performed based on Lee et al. (2002) (Lee, Kim, Kim, & Jang, 2002). A volume of 5 µL of the extract (2 mg/mL) was carefully added to 2 µL of pGEM®-T plasmid DNA (0.5 µg/well). The blends were kept for 10 min at room temperature before 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 then incubated for 5 min at 37 °C. DNA was analysed on 1% (w/v) agarose gel by electrophoresis and visualized under UV light.

All findings were estimated as EC_{50} values (sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay) in mg per mL of extract.

2.6.2. The α-amylase inhibitory assay

The α-amylase inhibition assay was performed according to the method described by Deguchi et al., 2003 with slight modifications (Deguchi, Osada, & Watanuki, 2003). Briefly, the assay blend consisted of 500 µL of 1% starch solution, 400 µL of 0.1 M sodium phosphate buffer (pH 7.0), 50 µL of plant extract dissolved in dimethyl sulfoxide (DMSO) and 50 µL of pancreatic α-amylase (Sigma, St. Louis, USA) solution (2 U/mL). After incubation for 10 min at 37 °C, 3 ml of 3,5-dinitrosalicylic acid (DNS) color reagent were added. Lastly, the

solution was placed on a boiling water bath for 5 min, diluted with 20 mL of distilled water and the absorbance was read at 540 nm. The chard extract was tested for α-amylase inhibitory activity at different concentrations (10.0 - 0.15 mg/mL). A negative control sample was prepared as above, without adding the plant extract, while, acarbose was employed as a positive control. All data were expressed as percentage inhibition using the following equation:

$$(6)\ \text{Percentage inhibition} = ((A_{control} - A_{sample}) / A_{control}) \times 100$$

Where $A_{control}$ and A_{sample} are the absorbance values of the negative control and sample, respectively. IC_{50} value, defined as the sample concentration (mg/mL) at which 50% inhibition of the enzyme effect occurs, was measured from a graph plotting enzyme inhibition against sample concentration.

2.6.3. The α-glucosidase inhibitory assay

The α-glucosidase inhibition assay was performed according to the method described by (Casedas, Les, G-Serranillos, Smith, & López, 2017) with slight modifications. The α -glucosidase reaction mixture, containing 2.5 mM 4-*p*-nitrophenyl-α-D-glucopyranoside (4-*p*NPG), 250 µL of extract at different concentrations in DMSO and 0.3 U/mL of α-glucosidase in phosphate buffer (pH 6.9), was incubated at 37 °C for 15 min on a water bath. Tubes containing DMSO, enzyme and substrate were used as negative controls, while as positive controls Acarbose replaced the plant extracts. Absorbance of the resulting *p*-nitrophenol (pNP) was estimated at 405 nm and was considered directly representative of the enzyme activity. The chard extract was tested for α-glucosidase inhibitory activity at different concentrations (5.0 - 0.15 mg/mL). Percentage inhibitions for extracts and acarbose (I %) were expressed using the following formula:

$$(7)\ \text{Percentage inhibition (\%)} = ((A_{control} - A_{sample}) / A_{control}) \times 100$$

Where $A_{control}$ and A_{sample} are the absorbance values of the negative control and sample, respectively. The 50% inhibition concentration (IC_{50}, mg/mL) of chard extract against intestinal α-glucosidase was then calculated.

2.7. Statistical Analysis

All the assays were performed in triplicate. The data are expressed as mean values and standard deviation (SD). Differences between the different samples were evaluated using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 18.0.

3. Results and discussion

3.1. Nutritional properties

The knowledge of the nutritional compounds of edible species is essential to ascertain their suitability for human consumption. In this case, the nutritional profile of the studied Swiss chard leaves, expressed on a fresh weight (fw) basis, was evaluated and well-summarized in **Table 1**. Proximate composition showed that the moisture content of chard (93.35 g/100g fw) was comparable to that observed in previous studies for cultivated Chard (92.66 g/100g fw) and spinach beet, which belong to the same family, *Carpobrotus edulis* L. and *Spinacia oleracea* L. (91.52 and 86.00 g/100g fw, respectively). However, this moisture value was higher than the contents described for other comestible halophytes such as *Sporobolus virginicus* (L.) Kunth and *Salicornia bigelovii* L. (Alhadrami, Al-Shorepy, & Yousef, 2010; Lu et al., 2010). The protein (0.663 g/100 g fw) and ash contents (1.30 g/100 g fw) were also higher than those of *S. bigelovii* (Lu et al., 2010) While ash was analogous to the values mentioned for wild *Portulaca oleracea* (Aberoumand & Deokule, 2009). It is known that halophytic plants have higher ash contents than other comestible plants. In fact, their level is directly related to the total mineral content. Therefore, the high ash content measured for

chard leaves is very possibly related to the saline environment and to its ability to accumulate minerals (Rocha et al., 2017). The fat content, as expected was very low (0.099 g/100 g fw). This value is similar to those reported for other halophytes, such as *Arthrocnemum macrostachyum* (Moric.) C.Koch, *Sarcocornia perennis* (Mill.) A.J.Scott and *Salicornia ramosissima* J.Woods (Barreira et al., 2017) and is much lower comparing with cultivated Chard (0.20 g/100g fw, USDA, 2018). Total carbohydrates were the greatest abundant macronutrients, with 6.51 g/100g fw, followed by ash, proteins and fats. The total sugars content was 2.158 g/100g fw, which is slightly higher than the value reported for fennel (*Foeniculum vulgare* L.) leaves (1.29 g/100 g fw) (Barros, Carvalho, & Ferreira, 2010). Being sucrose was the main soluble sugar detected (1.11 g/100g fw), followed by mannose, glucose and inositol (**Table 2**).

The halophytes commonly contain appreciable levels of dietary fibers, which promote the development and protects the beneficial intestinal flora. In this study, dietary fiber content outcomes revealed that total dietary fiber (TDF; **Table 1**) were 2.43 g/100g fw, being the insoluble dietary fiber (IDF) the predominant fraction (2.30 g/100g), with higher values comparing with it cultivated relative (1.6 g/100g fw; USDA, 2018). While, if we compared with other wild species such as *Beta maritima*, the TDF values of *Beta maritima* were twice more (4.38 g/100g fw), approximately (Tardío et al., 2016). Generally, the chemical composition of plant species differs depending on the harvest period and the growth conditions (e.g. climate, soil quality, irrigation, treatments, etc.). It is well-known that a daily intake of 7 g of plant dietary fibers is considered enough to significantly decrease the menace of cardiovascular and coronary heart diseases (Barreira et al., 2017). In this sense, the consumption of 100 g of fresh wild Swiss chard leaves would cover approximately 35% of the recommended daily dose. Thus, chard leaves can be considered an interesting source of dietary fiber and could be added to other foods to improve fiber intake.

Regarding mineral content, this fraction is one of the most interesting aspects influencing the use of edible vegetables in human nutrition. Minerals deficiency is one of the causes of numerous chronic and degenerative disorders. The micro minerals Fe, Cu, Mn and Zn play a major role in redox processes and are cofactors activating approximately 35 different enzymes. Fe was found in relative high amount (2.94 mg/100g fw), this content was higher than that reported for other edible wild leafy vegetables, such as *Beta maritima* (2.88 mg/100g fw), *Cichorium intybus* L. (1.29 mg/100g fw) or *Foeniculum vulgare* Mill (2.21 mg/100g fw) (García-Herrera et al., 2014; Guerrero, Madrid, & Isasa, 1999; Tardío et al., 2016) and higher comparing with it cultivated relative (1.80 mg/100g fw; USDA, 2018). This result is in good agreement with Bozokalfa et al. (Bozokalfa, Yağmur, Aşçıoğul, & Eşiyok, 2011) who underlined that Swiss chard of different origins and varieties have a high content of mineral compounds, especially iron. Iron has been considered one of the most plentiful elements on earth and it is an indispensable nutrient for humans. Hence, iron deficiency, the major reason of anemia, affects at least 2 billion people worldwide. More than half of iron-deficiency anemia cases may be averted by increasing the amount of iron in the diet. As the recommended daily Fe intake are about 8–10 mg/day for men and elderly women and about 16–20 mg/day for women below 50–55 years old (Sánchez-Mata & Tardío, 2016). The consumption of 100g of fresh Swiss chards leaves could cover around 18% and 37% of the Recommended Dietary Allowance (RDA) of this micronutrient. Moreover, this leafy vegetable can also be well-considered as iron sources to the human diet, based on the Regulation 1169/2011 (> 2.1 mg in 100 g of product as eaten).

Regarding macroelements composition of Swiss chard leaves, Mg, Ca and K were the most abundant (307.10, 154.10 and 70.25 mg/100g fw, respectively). Comparing with other wild plants, present higher amount of calcium and lower of potassium (67.1 and 988 mg/100g fw, respectively for *Beta maritima* leaves) (Tardío et al., 2016). On the other hand, Na was found

in a very low amount with 6.20 mg/100g fw (comparing with cultivated chard, 213 mg/100g fw, according to USDA, 2018; and other leafy vegetables and particularly other halophytes plants).

Although fat content and therefore, fatty acids relative percentage in leafy vegetables is not representative to energy value of this food products. Leafy vegetables presents a health fatty acids profile, rich in unsaturated fatty acids, such as oleic, linoleic and α-linolenic acids (Morales et al., 2012; Sánchez-Mata & Tardío, 2016). In this way, leaves of wild Swiss chard has been characterized through GC–MS analysis. Results are presented in **Table 3** and indicate a high content of linoleic acid, followed by a remarkable presence of oleic acid that is known to be very important in nervous cell construction. It has fundamental role in cardiovascular diseases prevention. Among the saturated fatty acids, the dominant component was palmitic acid (22.92%). A significant Unsaturated/saturated ratio (U/S) is regarded favourable for the reduction of serum cholesterol, atherosclerosis and prevention of heart diseases. These findings were quite similar to the fatty acid composition of chard seed oil after extraction with supercritical CO_2 (Ninfali & Angelino, 2013). Swiss chard leaves oil are rich in unsaturated fatty acids (monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA)). Additionally, this profile was in agreement with those described for other species belonging to the family of Chenopodiaceae such as *Sarcocornia* and *Salicornia* genera. Often, saturated fatty acids are common in halophytes and are probably related to the salt tolerance mechanisms. Indeed, the vacuolar membranes of *S*. *maritime* are composed by high levels of saturated fatty acids, which seem to contribute to the decreased membrane permeability to NaCl (Barreira et al., 2017).

3.2. Volatile compounds determination

The results for chard leaves volatile compounds are presented in **Table 4**. A total of 36 volatile components were detected. The identified components accounted for 95.1% of the

total aroma. Generally, each volatile compound is characterized by an odor threshold (varying from a few ppb to several ppm), so even if the qualitative composition of different samples is almost the same, the aroma may vary when the relative proportions are dissimilar (Visai & Vanoli, 1997). Quantitatively, the volatile profile displayed that non-terpene derivatives (40.6 %) which mainly straight-chain aldehydes as well as alcohols) and oxygenated monoterpenes (27.7 %) (alcohols, ketones, phenols and ethers) were found to be the main classes of volatiles in the chard leaves. Furthermore, phenylpropanoids were moderately present, with 11.3 %. This class of chemicals is reported to have a wide range of biological activities. The sesquiterpene hydrocarbons were scarcely represented (2.0 %). The main volatile constituents of the chard leaves were *(E)*-anethole (11.3 %), octanoic acid (7.5 %), decanal (7.3 %), α-terpineol (5.8 %) and limonene (5.6 %). Anethole has potent antimicrobial properties, against bacteria, yeast, and fungi (De, De, Sen, & Banerjee, 2002). The volatile fraction from the leaves of studied Swiss chard is qualitatively similar to that reported for the volatiles of leaves of *Beta vulgaris* subsp. maritima, even if in different proportion (Zardi-Bergaoui et al., 2017).

3.3. Bioactive compounds analysis

Furthermore, vegetables are an imperative source of phytochemicals, like carotenoids and chlorophylls, which have recognized health-promoting properties, e.g. antioxidant. Indeed, carotenoids are the most effective singlet oxygen quenchers and can also scavenge peroxyl radicals. Plants synthesize these phytochemicals to protect themselves from singlet oxygen produced by UV light. They are among the common natural pigments, and more than 600 different compounds are known, with β-carotene as the main one. (Trifunovic, Topalovic, Knezevic, & Vajs, 2015) As shown in **Table 1**, chard leaves exhibit low β-carotene and lycopene levels than other halophytes such as *Salicornia* and *Sarcocornia* species (Ventura et al., 2011). As expected, chlorophyll, although not principally significant in nutritional terms,

offered a measure of green vegetable color, an estimation of senescence for consumers and significant effects on oxidation, inflammation and wound healing (İnanç, 2011). Leaves of wild Swiss chard contain 0.21 mg/100 g fw of chlorophyll a and 0.06 mg/100 g fw of chlorophyll b. These values are comparable to those of Salicorniaceae halophytes (Barreira et al., 2017). Studies reported by Hsu et al. (Hsu, Chao, Hu, & Yang, 2013) has revealed that chlorophylls act, directly, as reducers of free radicals and have the capacity to protect lymphocytes against oxidative DNA damage by H_2O_2. Moreover, the natural chlorophylls prevent lipid peroxidation of LDL (Low Density Lipoproteins) (Hsu et al., 2013).

In this work, instead of other no-green chemistry solvents, ethanol was employed to extract dried Swiss chard leaves. The obtained extract was evaluated for total contents in phenolics (TPC), flavonoids (TFC), total flavonols, *o-diphenols* (O-PC) and condensed tannins (CTC). As reported in **Table 5**, TPC, TFC, total flavonols, O-PC and CTC reached 96.58 mg (GAE), 30.08 mg (CE), 22.69 mg (RE), 41.80 mg (CE) and 7.66 mg (HE) per g of dry extract, respectively. *O*-diphenols were noticed in lesser amounts than the other phenolic derivatives. Being natural extracts considered rich in phenolics when their TPC (expressed as GAE) is higher than 20 mg/g (Rocha et al., 2017), Swiss chard leaves can effectively be considered a promising source of those compounds. The TPC and TFC values were higher than those reported for other halophytes, e.g. *Arthrocnemum macrostachyum*, *Sarcocornia perennis alpini*, *Sarcocornia perennis perennis* and *Salicornia ramosissima*. Halophytes live in very harsh environments, with high UV radiation and salinity levels, and these stressful conditions often lead to the creation of radical oxygen species (Bose, Rodrigo-Moreno, & Shabala, 2014). As a defense, halophytic plants probably produce antioxidant phenolic compounds as a response to oxidative stress.

Moreover, to further evaluate the individual phenolic components present in the extract, sample was analyzed by HPLC-DAD and results are summarized on **Table 5.**

In the Chenopodiaceae family, some phenolic compounds are abundant (Boulaaba et al., 2013). In the present study, hydroxycinnamic acids (coumaric and rosmarinic) and myricitrin acids were identified through a comparison with the retention time of authentic standards analyzed under identical conditions as shown in **Fig. S1** (peak 1: 19.7 min, peak 2: 20.7 min and peak 3: 21.5 min) as p-Coumaric, myricitrin and rosmarinic acid derivatives), which is in arrangement with other reports (Ninfali & Angelino, 2013). Thus, myricitrin acid was the major identified phenolic acid (4.08 mg/g extract) followed by p-Coumaric (3.53 mg/g extract). This finding was approximately similar to those reported by Rocha et al, 2017 for *Carpobrotus edulis* extract (Rocha et al., 2017). It was widely reported that phenolic acids are responsible for the antioxidant capacity (potent redox properties) and several biological activities (Bennett et al., 2003; Bogucka-Kocka, Zidorn, Kasprzycka, Szymczak, & Szewczyk, 2016; Nouman et al., 2016).

3.4. Bioactive properties

3.4.1. Antioxidant activity evaluation

Essential oil derived from halophytes have been proved to possess potent antioxidant activities (Jallali et al., 2014). Polyphenols are well-known for their antioxidant capacity as radical scavengers and possible beneficial roles in human health, such as reducing the risk of cancer, cardiovascular disease, and other pathologies (Sacan & Yanardag, 2010). Plants containing high phenolic components can be a main source of antioxidants (Barros, Morales, Carvalho & Ferreira, 2016). For these reasons, the antioxidant potential of the ethanol extract prepared from chard leaves was evaluated by five complementary techniques, including its ability to scavenge the free radicals $DPPH^{\bullet}$ and $ABTS^{+\bullet}$, its power to reduce iron (III), its capacity to prevent bleaching inhibition of β-carotene and to inhibit linoleic acid peroxidation as well as its aptitude to protect DNA against Fenton's reagent. As reported in **Table 6**, the

ethanol extract presents very outstanding antioxidant activities on $DPPH^{\bullet}$ (EC_{50}= 0.75 mg/mL), $ABTS^{+\bullet}$ (EC_{50}= 1.22 mg/mL), reducing power (EC_{50}= 0.21 mg/mL), β-carotene bleaching (EC_{50}= 0.10 mg/mL) and TBARS (EC_{50}= 0.08 mg/mL). Herein, the antioxidant activities of the ethanol extract may be ascribed to it high of phenolic contents. Antioxidant activity of phenolics is due to the existence of a hydroxyl group which well-donates protons to free radicals and scavenge them. These results are in good agreement with previous studies recently reviewed (Ninfali & Angelino, 2013). Moreover, the antioxidant capacity of the chard extract was also assessed using the DNA nicking trial. Results are illustrated in **Fig. 1**, where line 3 represents the native DNA (the untreated plasmid) with its two forms: the nicked as well as the supercoiled form, while line 2 showed the plasmid DNA incubated with Fenton's reagent in the absence of chard extract. In the latter case, incubation resulted in the complete degradation of the supercoiled form. On the contrary, in presence of the chard extract (line 1), a notable protection effect against hydroxyl radical induced DNA damage is apparent. These results confirm Swiss chard as an interesting source of antioxidants.

3.4.2. α-Amylase and α-glucosidase inhibitory activities

Diabetes is recognized as one of the major causes of morbidity and mortality in the world. In fact, about 2.5–3% of the world's population suffers from this disease, a portion which in some countries reaches 7% or more. Diabetes is a metabolic disorder characterized by hyperglycemia. It damages major organs, comprising the lungs, kidneys, testes, heart, vessels, and eyes, through disrupted glycemic control and increased inflammation (Gezginci-Oktayoglu et al., 2014; Ozsoy-Sacan, Karabulut-Bulan, Bolkent, Yanardag, & Ozgey, 2004). Diabetes is commonly caused by a number of lifestyle-related risk factors including smoking, obesity, physical inactivity and poor diet (Boath, Stewart, & McDougall, 2012), and may be managed by using drugs to delay or prevent the absorption of glucose from meals. The digestive enzymes, α-amylase and α-glucosidase, are the key enzymes participating in the

breakdown of carbohydrate into glucose before its subsequent uptake into the bloodstream. The usually used drugs for providing inhibition of enzymes include miglitol, and acarbose; however, these drugs can cause side-effects like abdominal flatulence, discomfit and diarrhea, which reduce patient compliance and treatment effectiveness (Pantidos et al., 2014). Thus, it is needed to explore natural inhibitors that could replace these drugs.

Scientific reports about the antidiabetic properties of plant extracts through the inhibition of carbohydrate-hydrolysing enzymes have significantly increased during the past few decades. *Beta vulgaris* is one of the medicinal herbs that could be employed by diabetics (Ozsoy-Sacan et al., 2004). In our study, the ethanol extract prepared from chard leaves revealed significant inhibitory effects on α-glucosidase (IC_{50}= 0.13 mg/mL) and α-amylase (IC_{50}= 1.03 mg/mL) activities, as summarized in **Table. 6**. The mechanism for the enzymes inhibitory effect of the extract has been possibly attributed to saponins, that inhibit gluconeogenesis and glycogenolysis (Massiot et al., 1994). However, other molecular pathways potentially involved in the enzymes inhibitory effects must be further investigated. In fact, it was recommended that the enzymes inhibitory capacity of Swiss chard extract may be due to its content in flavonoids, via the inhibition of glucose transporters. For instance, quercetin displayed evidence of anti-diabetic effects via inhibition of the intestinal glucose transporter GLUT2 (Song et al., 2002). Other complementary mechanism could be the flavonoid induced inhibition of the α-amylase and α-glucosidase activities. For instance, two flavonolglycosides isolated from *Salsola kali* were demonstrated to be active inhibitors of α-amylase (Tundis, Loizzo, Statti, & Menichini, 2007). The inhibition of this enzyme could delay the digestion and absorption of carbohydrates and consequently suppress postprandial hyperglycemia. Some C-glycosyl flavones, such as vitexin, isovitexin, orientin and isoorientin, contained in Swiss chard leaves and seeds, were found to inhibit α-glucosidase and might be the most probable responsible for the enzyme inhibitory activity evidenced in diabetic (Li et al., 2009).

4. Conclusions

The present paper describes the nutritional values (including proximate composition, dietary fibers, fatty acids and the mineral elements profile) of wild Swiss chard leaves, an edible halophyte medicinal plant, to determine its potential as an alternative green leafy vegetable for human consumption. Results proved that chard leaves have a nutritional profile suitable to be including in modern diets. Also, chard leaves exhibit a significant content of phytochemicals and antioxidants compounds, such as flavonoids, phenolic acids (Myricitrin, p-cumaric acid and rosmarinic acid), pigments (chlorophyll, β-carotene and lycopene) and some volatile compounds (*(E)*-anethole (11.3 %), octanoic acid (7.5 %) and decanal (7.3 %)). Furthermore, its ethanol extract showed a considerable potential for the use in phytotherapy. Indeed, it exhibited very good antioxidant abilities on $DPPH^{\bullet}$, $ABTS^{+\bullet}$, reducing power, TBARS, β-carotene inhibition and DNA nicking assays, together with relevant inhibitory effects on α-amylase and α-glucosidase. Our data revealed that Swiss chard extract could be explored as a promising functional food ingredient and/or nutraceutical with antioxidant and anti-hyperglycemic potential. Altogether our findings demonstrate the importance nutritional value of Swiss chard and the extent of nutritional diversity. This valuable information suggests that the consumption of leaves from Swiss chard can contribute for a balanced diet and may use in modern diets, as a dietary supplement or as functional ingredient in the design of novel food products.

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Table 1. Nutritional and chemical composition (mean ± SD, n = 3) of wild Swiss chard leaves.

		Chard leaves
Proximate composition (g/100 g fw)	Moisture	93.35 ± 0.29
	Fat	0.099 ± 0.01
	Proteins	0.663 ± 0.01
	Ash	1.30 ± 0.06
	Total available carbohydrates	2.158 ± 2.8
	Total dietary fiber (TDF)	2.43 ± 0.29
Dietary fiber composition (g/100 g fw)	Insoluble dietary fiber (IDF)	2.30 ± 0.37
	Soluble dietary fiber (SDF)	0.13 ± 0.00
Mineral composition (mg/100 g fw)	Cu	0.15 ± 0.02
	Fe	2.94 ± 0.03
	Mn	0.32 ± 0.00
	Zn	0.30 ± 0.09
	Ca	154.10 ± 1.09
	Mg	307.10 ± 42.89
	Na	6.20 ± 0.33
	K	70.25 ± 0.84
Pigments composition (mg/100 g fw)	Chlorophyll a	0.21 ± 0.00
	Chlorophyll b	0.06 ± 0.001
	Total chlorophylls	0.28 ± 0.02
	β-carotene	0.05 ± 0.003
	Lycopene	0.02 ± 0.00

Table 2. Soluble sugars composition of wild Swiss chard leaves (g/100g fw).

	Swiss chard leaves
Soluble sugars	
Fructose	0.046 ± 0.01
Glucose	0.285 ± 0.02
Galactose	0.038 ± 0.01
Arabinose	0.003 ± 0.01
Rhamnose	0.018 ± 0.04
Sucrose	1.115 ± 0.05
Raffinose	0.061 ± 0.01
Polyols	
Inositol	0.182 ± 0.02
Mannitol	0.082 ± 0.02
Total soluble sugars	2.382 ± 0.08
Total polyols	0.264 ± 0.02
Sum of sugars	2.646 ± 0.04

Table 3. Fatty acids profile (relative percentage, %) of wild Swiss chard leaves.

Fatty acids		**Swiss Chard**
Caproic acid	C6:0	0.37 ± 0.01
Lauric acid	C12:0	2.17 ± 0.10
Myristic acid	C14:0	0.14 ± 0.01
Pentadecanoic acid	C15:0	0.79 ± 0.01
Palmitic acid	C16:0	22.92 ± 1.23
Margaric acid	C17:0	1.54 ± 0.03
Stearic acid	C18:0	8.17 ± 0.09
Arachidic acid	C20:0	3.82 ± 0.05
Behenic acid	C22:0	0.67 ± 0.12
Tricosanoic acid	C23:0	0.81 ± 0.01
Lignoceric acid	C24:0	0.16 ± 0.01
Palmitoleic acid	C16:1	6.31 ± 1.03
Oleic acid	C18:1	19.15 ± 1.15
Eicosenoic acid	C20:1	0.63 ± 0.01
Linoleic acid	C18:2 n6	26.54 ± 2.02
Eicosadienoic acid	C20:2	0.65 ± 0.01
α-Linolenic acid	C18:3 n3	5.17 ± 0.06
SFA		41.56 ± 1.34
MUFA		26.09 ± 1.01
PUFA		32.36 ± 0.03
U/S		1.40 ± 0.01

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; U/S: Unsaturated/saturated ratio.

Table 4. Volatiles (%) of the wild Swiss chard leaves.

N°	Constituents	LRI	Swiss chard leaves (%)
1	Isovaleric acid	836	1.5
2	α-pinene	941	1.9
3	Benzaldehyde	963	0.5
4	β-pinene	982	1.8
5	6-methyl-5-hepten-2-one	987	2.6
6	Limonene	1032	5.6
7	*(E)*-3-octen-1-ol	1062	2.7
8	Acetophenone	1068	0.8
9	*Cis*-linalool oxide (furanoid)	1076	2.1
10	*n*-undecane	1100	0.5
11	Linalool	1101	0.9
12	Nonanal	1104	3.0
13	Methyl octanoate	1128	2.5
14	Camphor	1145	1.2
15	Menthol	1174	0.8
16	Octanoic acid	1181	7.5
17	α-terpineol	1191	5.8
18	*Cis*-dihydrocarvone	1195	2.3
19	*n*-dodecane	1200	4.8
20	Decanal	1206	7.3
21	Verbenone	1207	0.9
22	β-cyclocitral	1222	2.6
23	Citronellol	1228	2.8
24	*Exo*-fenchyl acetate	1230	2.1
25	Carvone	1244	2.4
26	Geraniol	1256	3.0
27	3-methyldodecane	1273	0.9
28	*(E)*-anethole	1285	11.3
29	*n*-tridecane	1300	1.5
30	Methyl decanoate	1327	2.1
31	α-terpinyl acetate	1352	2.2
32	*n*-tetradecane	1400	1.5
33	β-caryophyllene	1419	2.0
34	Δ8,9-dehydro-4-hydroxythymol dimethyl ether	1444	1.2
35	*(E)*-β-ionone	1487	1.6
36	*n*-pentadecane	1500	0.9
	Monoterpene hydrocarbons		9.3
	Oxigenated monoterpenes		27.7

Sesquiterpene hydrocarbons	2.0
Phenylpropanoids	11.3
Apocarotenoids	4.2
Non-terpene derivatives	40.6
Total identified (%)	**95.1**

LRI: linear retention indices on DB-5 column.

Table 5. Phenolic composition of wild Swiss chard ethanolic extract.

		Leaves ethanolic extract
Phenols composition	*p*-coumaric acid[a]	3.53
	Myricitrin acid[a]	4.08
	Rosmarinic acid[a]	1.02
	Total Phenolics content[b]	96.58 ±1.81
	Total Flavonoids contenc[c]	30.08 ± 1.02
	Total Flavonols content[d]	22.69 ± 1.31
	Total Tannins content[e]	41.80 ± 11.27
	Total orthodiphenols content[f]	7.66 ± 0.02

[a]: Identification according to their retention time; mg/g extract.
[b]:mg GAE/g extract: mg of Gallic Acid Equivalents (GAE) per g of extract.
[c]:mg CE/g extract: mg of Catechin Equivalents (CE) per g of extract.
[d]:mg RE/g extract: mg of Rutin Equivalents (RE) per g of extract.
[e]: mg CE/g extract: mg of Catechin Equivalents (CE) per g of extract.
[f]:mg HE/g extract: mg of Hydroxytyrosol Equivalents (HE) per g of extract.

Table 6. Antioxidant, α-amylase, α-glucosidase inhibitory activities (EC_{50} values for antioxidant assays and IC_{50} values of Swiss chard leaves ethanol extract against α-amylase and α-glucosidase).

Antioxidant activity (EC_{50}, mg/mL)	$DPPH^{\bullet}$ scavenging ability	0.75 ± 0.07
	$ABTS^{+\bullet}$	1.22 ± 0.52
	Reducing power	0.21 ± 0.03
	β-carotene bleaching inhibition	0.10 ± 0.01
	TBARS inhibition	0.08 ± 0.01
(IC_{50} values, mg/mL)	**α-Glucosidase inhibitory activity**	
	Ethanol extract	0.13 ± 0.12
	α-Amylase inhibitory activity	
	Ethanol extract	1.03 ± 0.08

EC_{50}: Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. Mean ± SD, n = 3. Means ± SD, n=3. α-amylase and α-glucosidase inhibitory activity of control (acarbose): 0.23 mg/mL for α-Glucosidase and 0.08 mg/mL for α-Amylase

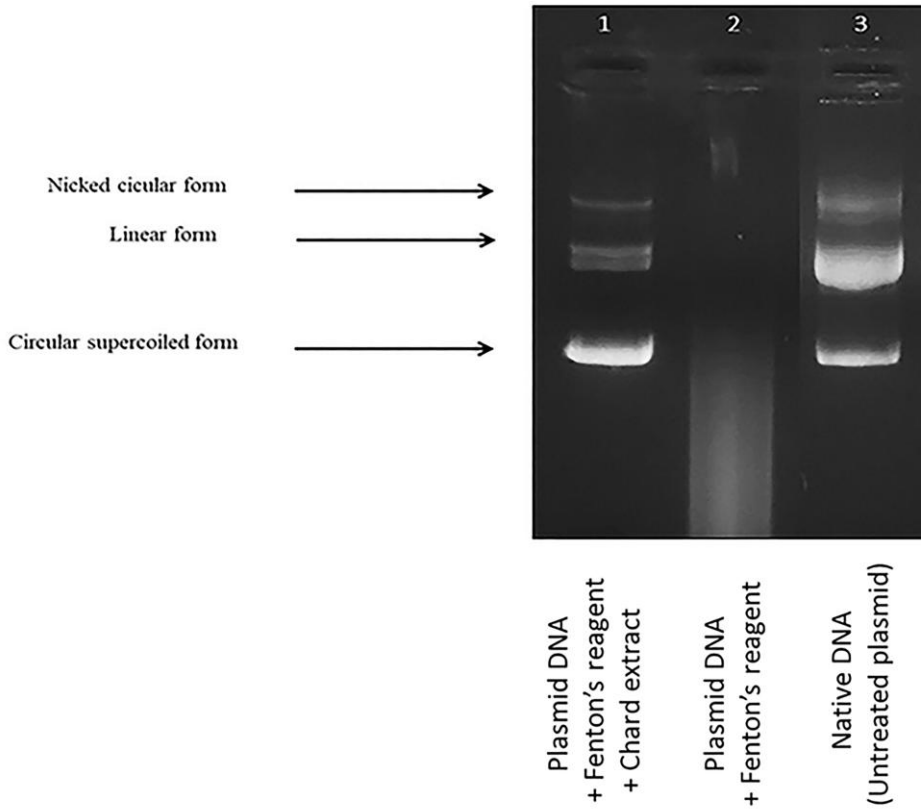

Figure 1. Gel electrophoresis pattern of the plasmid pGEM®-T incubated with Fenton's reagent in the presence and absence of chard ethanolic extract.

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*Graphical Abstract

Graphic abstract legend

Nutritional, phytochemical composition and bioactivity (antioxidant, hypoglycemic and antimicrobial activity) of Edible Swiss chard (*Beta vulgaris* L. var. cicla) were investigated.