



Nutritional value and functional properties of an underexploited Tunisian wild beet (*Beta macrocarpa* Guss.) in relation to soil characteristics

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Abstract

The study centers on the valorization of *Beta macrocarpa* Guss., an endangered Mediterranean wild plant that grows in Tunisia. This plant is disappearing due to a reduction in marginal areas and a lack of awareness of this important crop wild relative (CWR). This prompted us to carry out work to assess the nutritional and functional value of its plant shoots in relation to physicochemical soil properties at three different Tunisian sites covering the north (Sijoumi), the center (Enfidha) and the south (Kerkennah) of the country. All soil samples showed an alkaline pH and high salinity. Sijoumi, Enfidha and Kerkennah soils were classified as loamy, silty clay loamy and sandy, respectively. Chemical analysis revealed that all soils, especially the sandy one, were low in total nitrogen, organic matter and microelements. Plant analysis showed that shoots harvested from the loamy soil presented the highest levels of carbohydrate (19.1 g/100 g FW) and fiber (6.1 g/100 g FW) and the greatest energetic value (94 kcal/100 g FW), whereas shoots collected from the sandy soil showed the highest contents of protein (4.1 g/100 g FW), ash (5.2 g/100 g FW), total polyphenols and flavonoids (39.01 mg GAE/g DW; 27.8 mg CE/g DW), and the greatest DPPH scavenging capacity ($IC_{50} = 0.74$ mg/ml). The results suggest that *Beta macrocarpa*, which naturally grows in poor and salt-affected soils, could play a crucial role in maintaining the biodiversity and sustainability of agro-ecosystems, particularly in marginal areas, and could also provide an alternative source of food with significant nutritional value and health benefits.

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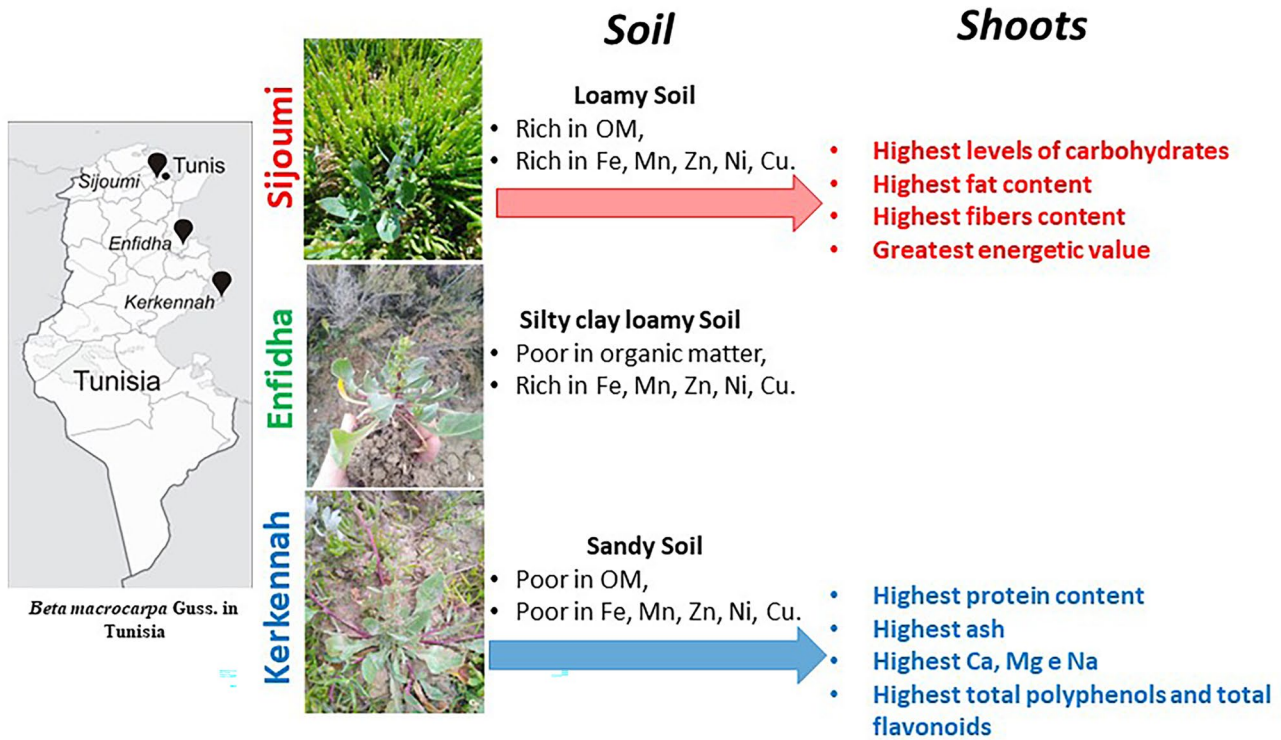
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Graphical abstract



Keywords CWR · Proximate composition · Minerals · Edaphic parameters · *Beta macrocarpa* · Total polyphenols

Introduction

Today, while scientists have different opinions on the origin of climate change, all of them agree that soil consumption, soil degradation and the loss of biodiversity along with the reduction in food production are due mainly to the development of a bad farming paradigm and, to some extent, the increase in the global human population. In any case, some scientists state that climate change is exerting considerable pressure upon agriculture and food production (Parry et al. 2004; Ray et al. 2015) at a time when an increase in food production of about 60% is needed to supply the increasing global population, which will reach 9.7 billion by 2050 (United Nations 2019). Warm temperatures and frequent periods of drought cause decreases in crop production (Long and Ort 2010) and quality (Dwivedi et al. 2013), and the scientific community from some countries in the Mediterranean Basin is focusing on this issue: it is attempting to identify the associated problems in order to find sustainable solutions, as in the project REstoration ACTions for the MEDiterranean (REACT4MED) (<https://react4med.eu>). Moreover, climate change also affects the distribution and

virulence of crop pests and diseases, which constitute an additional threat to agricultural production (Patz and Kovats 2002; McMichael et al. 2006). This will have a considerable impact on agricultural sustainability and food security, which is especially important given that around 800 million people are already suffering from food insecurity and malnutrition (FAO 2014). To cope with these negative impacts of climate change which our world is facing nowadays, we need to develop a more resilient agriculture system that is capable of ensuring food security over the coming decades (Gasparini et al. 2021). Thus, the exploration of new plant resources with significant nutrition potential and a high tolerance to climatic change should be considered.

One alternative source of food is wild edible plants (WEPs), which are naturally adapted to a wide range of soils and climates and can survive in hard environmental conditions (Zait and Schwartz 2018). They constitute a valuable reservoir of nutritious and healthy foods: they are rich in minerals, vitamins and fiber (Baldermann et al. 2016) as well as in plant secondary metabolites, i.e., alkaloids, polyphenolic compounds and essential fatty acids, and they also possess some medicinal properties, including

anti-bacterial, hepatoprotective and anti-carcinogenic activities (Heywood 1999). For millennia, WEPs have supported food security (Addis et al. 2005) and have alleviated malnutrition and poverty in rural communities of the Mediterranean Basin (Maxted and Vincent 2021), where farming is the main source of food and income (Baldermann et al. 2016; Harisha et al. 2021). Even today, they are used as additional sources of healthy food, bioactive compounds and medicine (Keller et al. 2005; Termote et al. 2011; Ulian et al. 2020; Casella et al. 2023). Among WEPs, crop wild relatives (CWRs) constitute an important category of wild edible plants (Maxted and Kell 2009; Perrino et al. 2014; Perrino and Calabrese 2014; Maxted et al. 2006; Pinela et al. 2017; Perrino and Perrino 2020; Perrino and Wagensommer 2021; Accogli et al. 2023) that are closely related to cultivated species of socio-economic value (Maxted et al. 2006) and can be used to improve tolerance and/or resistance to biotic and abiotic stresses and to enhance production in terms of quantity and quality (Redden 2015).

Beta macrocarpa is an annual CWR species that belongs to the Chenopodiaceae family (Pottier Alapetite 1979) and is closely related to sugar beet crops (El-Mokni et al. 2022). It is widespread in inland or coastal habitats in western and eastern Mediterranean areas (Hessini et al. 2020). In Tunisia, one of the most important hotspots of CWR diversity in the Mediterranean Basin, this species is exclusively found in salty ecosystems, precisely at the edge of sebkhas, among halophyte tufts (Abdelly 1997). It is a salt-tolerant plant that can withstand high soil salinity through morphological, structural and functional adaptations (Hamouda et al. 2016), and so it could potentially be used to valorize the salt-affected soils in marginal areas (Abdelly 1997; Barreira et al. 2017). In some rural regions of Tunisia, this wild beet is considered part of the local food tradition and consumed as cultivated vegetables (Dop et al. 2020).

Despite the significant economic, social and ecological benefits that *B. macrocarpa* can offer, and the fact that is still traditionally consumed by local communities, it remains underexploited. This is due to a lack of knowledge of its nutritional value and health benefits (Ratnayake et al. 2021; Nirmala et al. 2022). An analysis of the plant's proximate and mineral composition and its functional properties could enable it to be recommended as part of a balanced modern diet, as a food supplement or as a functional ingredient for large-scale human consumption.

The present study aimed to (i) evaluate the proximate and mineral composition as well as the functional properties of *Beta macrocarpa* growing in different pedo-climatic areas in relation to soil characteristics and (ii) highlight its potential as a new healthy and resilient food source with high nutritional value. To the best of our knowledge, this is

the first study to assess the nutritional profile and functional properties of *Beta macrocarpa* shoots growing in different regions of Tunisia in relation to edaphic factors.

Material and methods

Sampling sites

The sampling of soil and plants was carried out in three different sites (Fig. 1a, b). The first site was the Sebkhia of Sijoumi (36° 75' 69.51" N, 10° 12' 22.26" E), located in the north of Tunisia (Tunis Governorate), which is characterized by a semi-arid climate in its temperate winter ($P = 450$ mm, annual $T = 18.6$ °C); the second one was situated in the center of the country, in Sebkhia of Enfidha (36° 05' 31.5" N, 10° 27' 23.5" E) (Sousse Governorate), which is also characterized by a semi-arid climate in its temperate winter ($P = 339$ mm, annual $T = 19.5$ °C); and the third site was located in the south, more exactly in the Kerkennah Islands (34° 70' 21.02" N, 11° 14' 84.65" E) (Sfax Governorate), which are characterized by an arid climate in their warm winter ($P = 195$ mm, annual $T = 25$ °C).

Soil analysis

For soil analysis, 1 kg of soil was taken at 20–30 cm depth at each site and then dried in the air at ambient temperature, ground, sieved and stored until analysis in the laboratory. The soil texture was determined through its particle size distribution determined via the pipette method (Piper 1947; El-Amier et al. 1995), and the textural class was attributed according to the USDA classification. The pH of the soil was measured using the filtrate obtained from a soil suspension in distilled water (1:2.5) by using a pH meter. The electrical conductivity (EC) was determined on an aqueous soil extract (1:2 w/v) using a conductometer and expressed in dS/m. Among the chemical properties, the determination of the total carbonate CaCO_3 percentage (%) was achieved using the gas volumetric method and a Dietrich–Fruhling calcimeter to quantify the CO_2 evolved in a closed system after HCl treatment (Leogrande et al. 2021). The total nitrogen was determined through the modified Kjeldahl method proposed by Bremner (1996). In order to evaluate the available phosphorus in the soil, the method of Olsen and Sommers (1982) was used. The organic carbon was determined by the Walkley–Black method described by Nelson and Sommers (1996). The amount of organic matter in the soil was calculated according to the following formula: organic matter (%) = organic carbon (%) $\times 1.724$. The exchangeable cations (Na^+ , Ca^{2+} , Mg^{2+} and K^+) were determined by using BaCl_2 (1 M) and

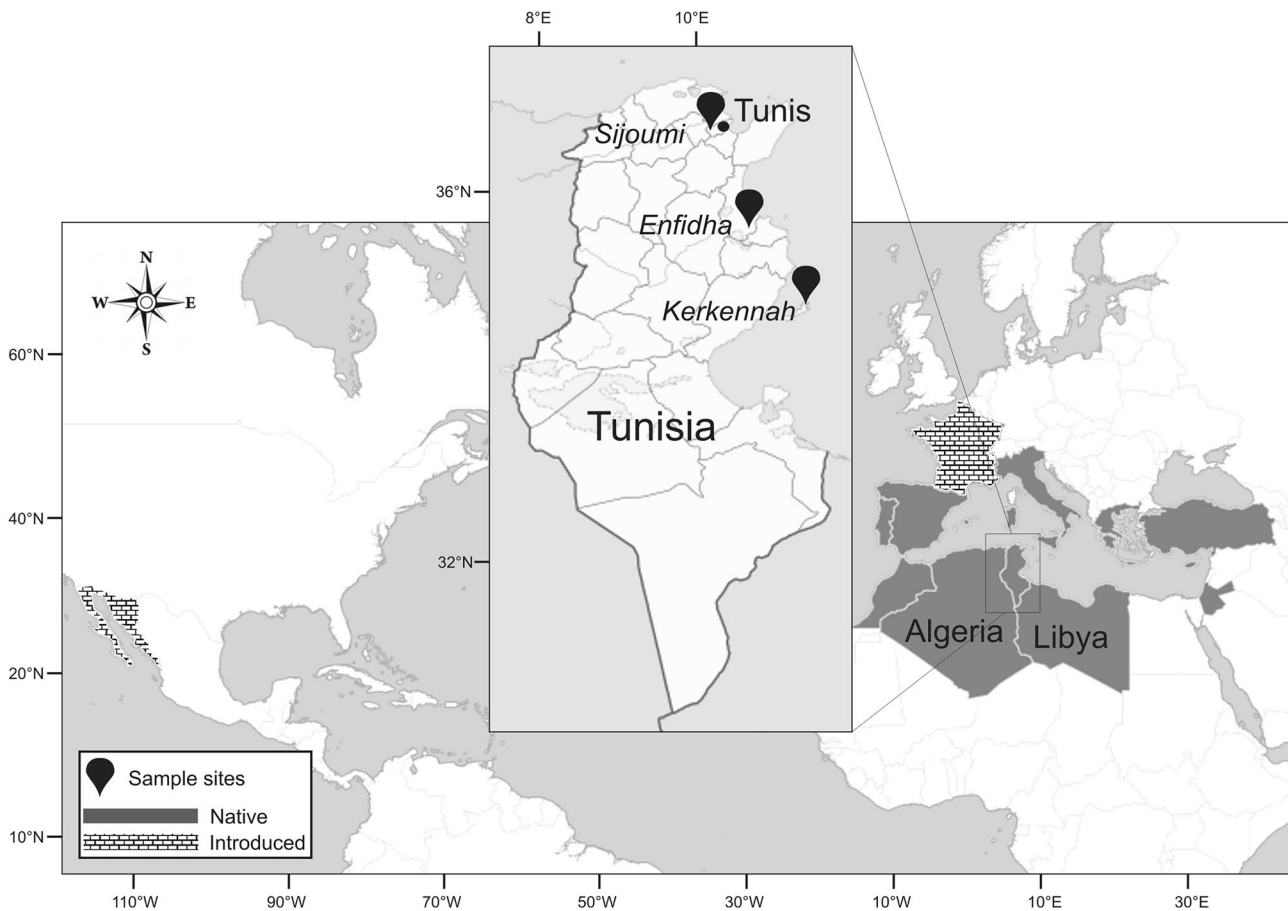


Fig. 1 **a** Geographical map showing the three sites (Sijoumi, Enfidha and Kerkennah) in which soil and plant sampling was performed. **b** Geological map of Tunisia

triethanolamine buffered at pH 8.1 as an exchange solution. The assessment of cations was achieved using inductively coupled plasma atomic emission spectroscopy (ICP-OES). The microminerals (Fe, Mn, Cu, Zn and Ni) were extracted by DTPA (5 mM), CaCl_2 (10 mM) and triethanolamine buffered at pH 7.3. The determination of available microminerals was carried out by using ICP-OES.

Plant material

The *Beta macrocarpa* (Guss.) plant specimens (Fig. 2) from three populations belonging to the same sites (Sijoumi, Enfidha and Kerkennah) in which soil sampling was performed as described above were collected at the flowering stage (March–April 2020). The harvested plants were authenticated in situ according to the morphological characters reported by Pottier Alapetite (1979) in *Flore de la Tunisie*, and the voucher specimens were deposited in the herbarium of the Tunisian National Institute of Agronomic

Research (INRAT). For plant analysis, the roots were removed from the freshly collected plants, and the aerial parts (stems, leaves and flowers) were washed with tap water, rinsed with distilled water and dried in a ventilated oven at 50 °C for 24 h. After that, the dried samples were ground into powder using a grinder and kept at room temperature until further use (Piscitelli et al. 2015; Perrino et al. 2021).

Plant analysis

Proximate composition

The proximate composition, including moisture, crude proteins, carbohydrates, fat, fiber, ash and energy, was determined using the official Association of Official Agricultural Chemists (AOAC 1990) methods. The moisture, which corresponds to the weight loss upon drying the plants in the

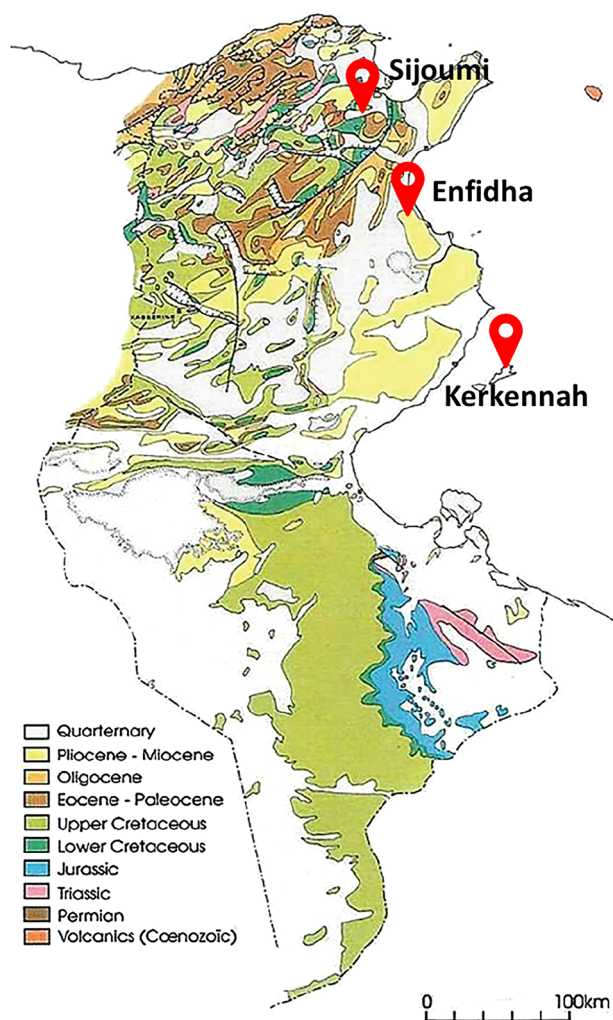


Fig. 1 (continued)

oven, was determined from the fresh (FW) and dry weights according to the following formula:

$$\text{Moisture (\%)} = (\text{FW} - \text{DW}) / \text{FW} \times 100.$$

Total protein content was estimated from the total N content measured following the micro Kjeldahl method. The ash content was obtained through sample incineration at 550 °C. The crude fat was extracted with petroleum ether using a Soxhlet apparatus and determined according to the method of Señoráns and Luna (2012). The crude fiber was measured by treatment of the sample with 1.25% H₂SO₄ and 1.25% NaOH, with filtering and washing with hot water performed after each step. After digestion, the residue obtained was dried in an oven at 105 °C ± 2 °C for 2 h and ashed at 550 °C in a furnace. The loss of weight on ignition was expressed as the content of crude fiber. The total carbohydrate value was given by 100% (percentage of ash + percentage of fat + percentage of protein). All results were expressed in g/100 g (DW). The energetic value was calculated by multiplying the total carbohydrate, lipid and protein values by factors of 4, 9 and 4, respectively, summing the products, and expressing the result in kilocalories (kcal) (Guil-Guerrero et al. 1998).

Mineral composition

The mineralization of the samples was carried out using a microwave mineralizer. For mineral element analysis, about 0.3 g of each sample was digested with 6 ml of concentrated HNO₃ and 1 ml of 30% H₂O₂, and this mixture was left to react overnight. After digestion, the analysis of macroelements (Ca, Mg, K and Na) and microelements (Zn, Cu, Mn, Fe and Ni) was carried out by ICP-OES. The colorimetric method of Murphy and Riley (1962) was used for the determination of phosphorus in mineralized samples. Results were expressed in mg/100 g (FW).

Fig. 2 *Beta macrocarpa* (Guss.) plants collected at the flowering stage from Sijoumi (a), Enfidha (b) and Kerkennah (c)



Total phenolic compounds and total flavonoid content

The total polyphenol contents of the methanolic extracts of all samples were determined by the Folin–Ciocalteu method adapted by Dewanto et al. (2002). Briefly, 125 ml of each extract was diluted with 500 ml of distilled water and 125 ml of the Folin–Ciocalteu reagent was added. This mixture was allowed to stand for 3 min before 1250 ml of Na_2CO_3 (7%) was added. The solution was then incubated in the dark for 90 min before measuring the absorbance at 760 nm in a UV–visible spectrophotometer (T60). Total polyphenol contents of extracts were expressed in mg gallic acid equivalent per gram (mg GAE/g DW) using a calibration curve with gallic acid (0–500 mg/ml). The total flavonoid contents of the extracts were determined using the aluminum chloride colorimetric method (Dewanto et al. 2002). In this, 250 ml of extract was mixed with 75 ml of NaNO_2 (5%). Next, 150 ml of AlCl_3 (10%) and 500 ml of NaOH (1 M) were added. The absorbance was measured at 510 nm. The total flavonoid content was calculated using the calibration curve for catechin (0–500 mg/ml) and was expressed in mg of catechin equivalent per g of dry weight (mg CE/g DW).

DPPH scavenging assay

The capacity of the extracts to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) was investigated via the procedure formerly described by Ben Mahmoud et al. (2022). For that, 50 ml of a particular concentration of ascorbic acid (five concentrations in the range 0.5–5 $\mu\text{g}/\text{ml}$ were tested) or extract (five concentrations in the range 0.5–4 mg/ml were tested) was mixed with 250 ml of an ethanolic solution of DPPH (8.66×10^{-5} M), and these were allowed to react in the dark for 30 min. Then, the absorbance of the resulting solution was read at 517 nm. The antiradical activity was determined using the following formula:

$$\text{DPPH scavenging activity (\%)} = (\text{Abscontrol} - \text{Abssample}) / \text{Abscontrol} \times 100,$$

where Abscontrol is the absorbance of the control (DPPH solution without the extract) and Abssample is the absorbance of DPPH solution with the extract. The antiradical activity was expressed as the IC_{50} value (mg/ml)—the extract dose required to cause a 50% decrease in the absorbance at 517 nm. Ascorbic acid was used as a positive control for comparison.

Statistical analysis

All measured data were reported as the mean \pm standard deviation of triplicate measurements. Significant differences ($P < 0.05$) between means were analyzed by ANOVA and the

Duncan test using the program package Statgraphics Centurion version 18.1.01. The Duncan test, at a 5% threshold, helps to group the individuals whose means for a particular parameter are not significantly different (Dagnelie 1975); it is slightly more conservative than the Fisher LSD test. The analysis of variance was performed for a 95% confidence interval (or 0.05) using the estimated means.

Results

In order to mitigate the impacts of climate change, to preserve ecosystems and to support the food security of the growing global population, we have to develop a sustainable agriculture system based on more resilient crops. The exploration of non-conventional crops, such as CWRs, with significant quality and high adaptability to climate change constraints is therefore highly recommended. In this context, the present work attempted to explore an edible CWR, *Beta macrocarpa*, as an alternative food source by analyzing its proximate and mineral composition and assessing its functional properties. Soil physicochemical parameters were also analyzed.

Taxonomy and nomenclature

The genus *Beta* L. belongs to the subfamily Betoideae and the family Chenopodiaceae. Currently, two sections are recognized in the *Beta* genus: *Beta* and *Corollinae*; the latter now includes the former section *Nanae* (Hohmann et al. 2006). The ten species of the section *Beta* are wind pollinators that are mainly distributed along European coasts. *B. adenensis* Pamukç. grows in some eastern Mediterranean areas in Greece and Turkey, while *B. corolliflora* Zosimovic ex Buttler, *B. lomatogona* Fisch. & C.A. Mey. and *B. macrorhiza* Steven are reported in Iran, the Transcaucasus and Turkey, *B. lomatogona* is found in Cyprus, and *B. macrorhiza* is found in the North Caucasus. *B. nana* Boiss. & Heldr., *B. patula* Aiton and *B. trojana* Pamukç. ex Aellen are endemic to Greece, two small islets of the Madeira Archipelago and Turkey, respectively. *B. trigyna* Waldst. & Kit. is reported in many countries, ranging from the former Yugoslavia to Turkmenistan and Ukraine. *B. palonga* R.K. Basu & K.K. Mukh. is native to Bangladesh, India and Pakistan (Powo 2023, <https://powo.science.kew.org/>). Finally, *B. macrocarpa* Guss., which is a primary wild relative of cultivated beets, is native to Algeria, the Balearic Islands, the Canary Islands, Cyprus, Greece, Italy, Crete, Libya, Morocco, Palestine, Portugal, Sardinia, Sicily, Spain, Tunisia and Turkey, while it was introduced to France and Northwest Mexico (Tutin et al. 1993; Dobignard and Chatelain 2011; Rebman et al. 2016).

B. macrocarpa is distinguished from *B. vulgaris* subsp. *maritima* (L.) Arcang. by some morphological characteristics (Letschert 1993). *B. vulgaris* subsp. *maritima* is characterized by a stem which is up to 80 cm long and procumbent to erect; its leaves are up to 10 cm long; its cymes have about 1–3 flowers; and its roots are usually not swollen. On the other hand, *B. macrocarpa* is characterized by basal leaves that are oblong-spathulate to triangular-ovate; its cymes have about 2–3 flowers; it has a pelviform receptacle and hard fruits, and the segments are up to 5 mm in size, erect, and often incurved at the apex (Tutin et al. 1993). Both diploid and tetraploid forms of the *Beta* section are found within *B. macrocarpa*, which is exclusively distributed in the Canary Islands. The tetraploid type is believed to be a natural amphidiploid hybrid between diploid *B. macrocarpa* and an unknown diploid of the *B. vulgaris* complex (Abe and Tsuda 1987). The diploid type of *B. macrocarpa* is distributed in the Mediterranean Basin, while the tetraploid type is endemic to the Canary Islands ($2n = 36$) (Lange and De Bock 1989).

Homotypic synonyms. *Beta vulgaris* var. *macrocarpa* (Guss.) Moq. in Chenop. Monogr. Enum. 14 (1840); *Beta vulgaris* subsp. *macrocarpa* (Guss.) Thell. in Mém. Soc. Sci. Nat. Math. Cherbourg 38:190 (1912).

Heterotypic synonyms. *Beta bourgaei* Coss. in Notes Pl. Crit. 44 (1849).

Conservation status. *B. macrocarpa* represents an area of occupancy (AOO) of less than 500 km² in Europe, with a discontinued distribution. It is therefore regionally assessed as being endangered (EN) (Bilz et al. 2011).

Soil properties

The results of the physicochemical analysis of the soils at the Sijoumi, Enfidha and Kerkennah sites are presented in Table 1.

The soil texture analysis revealed that Sijoumi soil consisted of clay (21%), silt (35%) and sand (43%); Enfidha soil was made up of clay (38%) and silt (50%); whereas Kerkennah soil was mainly sand (96%). According to the USDA classification, Sijoumi soil is classified as loamy, Enfidha soil as silty clay loamy and Kerkennah soil as sandy. With regards to pH and electrical conductivity (EC), statistical analysis ($P < 0.05$) showed that there was no significant variation; all soils displayed an alkaline pH (> 8), and high electrical conductivities of around 12, 25 and 32 dS/m were observed for the Kerkennah, Enfidha and Sijoumi soils, respectively (Table 1).

Chemical analysis results indicated that the total carbonate (TC), total nitrogen (TN), organic carbon (OC), organic matter (OM) and available phosphorus (P) contents were higher in Sijoumi and Enfidha soils than in Kerkennah soil. The TC

Table 1 Physicochemical properties of soil sampled from the Sijoumi, Enfidha and Kerkennah sites

Soil parameter	Sijoumi site	Enfidha site	Kerkennah site
Clay (%)	21 ± 8 ^b	38 ± 3 ^a	0.5 ± 0.1 ^a
Silt (%)	35.5 ± 11 ^b	50 ± 3 ^a	1 ± 0.1 ^c
Sand (%)	43.5 ± 18 ^b	13 ± 6 ^c	96.2 ± 0.1 ^a
Soil texture	Loamy	Silty clay loamy	Sandy
pH	8.5 ± 0.3 ^a	8.8 ± 0.4 ^a	8.9 ± 0.2 ^a
EC (dS/m)	32.1 ± 17.8 ^a	24.7 ± 24.2 ^a	12 ± 0.4 ^a
Total carbonate (%)	15.0 ± 7.8 ^a	12.0 ± 2.7 ^a	5.0 ± 1 ^a
Total nitrogen (g/kg)	1.1 ± 0.2 ^a	0.9 ± 0.5 ^a	0.5 ± 0.3 ^a
Organic carbon (g/kg)	18.6 ± 1.5 ^a	12.8 ± 3.8 ^b	7.3 ± 2.5 ^b
Organic matter (g/kg)	32.0 ± 3.1 ^a	22.0 ± 6.5 ^b	12.6 ± 4.7 ^b
Available P ₂ O ₅ (mg/kg)	25.4 ± 13.9 ^a	21.5 ± 14.7 ^a	6.4 ± 2.9 ^a
Exchangeable cations (mg/kg)			
K ⁺	61 ± 1 ^a	57 ± 1 ^a	63 ± 2 ^a
Ca ²⁺	753 ± 54 ^a	421 ± 21 ^a	581 ± 98 ^a
Mg ²⁺	3847 ± 11 ^a	5187 ± 15 ^a	2360 ± 67 ^a
Na ⁺	5 ± 1 ^a	89 ± 92 ^a	73 ± 76 ^a
Microelements (mg/kg)			
Fe	743 ± 1 ^a	742 ± 2 ^a	515 ± 10 ^b
Mn	178 ± 14 ^a	182 ± 7 ^a	25.9 ± 1.7 ^b
Zn	25.3 ± 1.6 ^a	25.1 ± 3.4 ^a	4.4 ± 0.1 ^b
Ni	10.6 ± 0.3 ^a	10.2 ± 0.3 ^a	< 0.001 ^b
Cu	0.92 ± 0.13 ^a	1.1 ± 0.18 ^a	< 0.001 ^a

Values are the means of three replicates ± standard deviation. Values with different superscripts (a, b and c) are significantly different at $P < 0.05$

level was about 15% and 12% in Sijoumi and Enfidha soils, respectively, against only 5% in Kerkennah. TN was found at levels of 1.1, 0.9 and 0.5 g/kg at the Sijoumi, Enfidha and Kerkennah sites, respectively. OC and OM contents differed significantly ($P = 0.01$) among the soil samples; the highest values (18.6 g/kg and 32 g/kg) were registered in Sijoumi soil. Available phosphorus (P) levels were about 21.5 and 25.4 mg/kg, respectively, in Enfidha and Sijoumi versus 6.4 mg/kg in Kerkennah. The data concerning exchangeable cations revealed that Mg²⁺ was the most abundant cation in all soils, particularly in Enfidha (5187 mg/kg); Ca²⁺ was more abundant in Sijoumi soil (753 mg/kg) than in Enfidha and Kerkennah soils. K⁺ (57–63 mg/kg) and Na⁺ (5–89 mg/kg) were present at the lowest levels. For microelements, an ANOVA test revealed a significant variation ($P < 0.01$) among the three analyzed soils (Table 1). Fe was the most abundant element in all soils (515–743 mg/kg), followed by Mn (25–142 mg/kg). Cu, Ni and Zn were detected in significantly smaller amounts. The lowest levels of all microelements were found in Kerkennah soil.

Table 2 Proximate and mineral compositions of *Beta macrocarpa* shoots collected from the Sijoumi, Enfidha and Kerkennah sites

Proximate composition	Sijoumi	Enfidha	Kerkennah
Moisture (%)	73.6 ± 2.5 ^b	85.4 ± 2.2 ^a	81.3 ± 1.2 ^a
Protein (g/100 g FW)	3.1 ± 0.9 ^a	2.4 ± 0.1 ^b	4.1 ± 0.3 ^a
Ash (g/100 g FW)	3.6 ± 0.7 ^b	2.8 ± 0.2 ^b	5.2 ± 0.7 ^a
Fat (g/100 g FW)	0.62 ± 0.2 ^a	0.27 ± 0.02 ^b	0.34 ± 0.04 ^b
Fiber (g/100 g FW)	6.1 ± 1.7 ^a	2.2 ± 0.4 ^b	1.5 ± 0.1 ^b
Carbohydrates (g/100 g FW)	19.1 ± 3.9 ^a	9.1 ± 1.9 ^b	9.1 ± 0.4 ^b
Energy value (kcal)	94 ^a	48 ^b	56 ^b
Minerals			
Na (mg/100 g FW)	856 ± 74 ^b	632 ± 9 ^c	1056 ± 96 ^a
K (mg/100 g FW)	385 ± 18 ^a	450 ± 82 ^a	598 ± 77 ^a
Ca (mg/100 g FW)	249 ± 70 ^a	101 ± 18 ^b	340 ± 48 ^a
Mg (mg/100 g FW)	206 ± 73 ^a	114 ± 4 ^b	256 ± 31 ^a
P (mg/100 g FW)	53 ± 17 ^a	50 ± 2 ^a	38 ± 6 ^a
Trace elements			
Fe (mg/100 g FW)	9.6 ± 5.5 ^a	5.1 ± 3.9 ^a	4.6 ± 1 ^a
Mn (mg/100 g FW)	2.3 ± 1 ^a	1.2 ± 0.3 ^a	0.7 ± 0.1 ^a
Zn (mg/100 g FW)	0.94 ± 0.32 ^a	0.55 ± 0.1 ^a	0.66 ± 0.1 ^a
Cu (mg/100 g FW)	0.22 ± 0.05 ^a	0.17 ± 0.03 ^a	0.22 ± 0.03 ^a
Ni (mg/100 g FW)	0.06 ± 0.01 ^a	0.06 ± 0.03 ^a	0.09 ± 0.04 ^a

Values are the means of three replicates ± standard deviation. Values with different superscripts (a, b and c) are significantly different at $P < 0.05$

Plant analysis

Proximate composition

The proximate composition, including the moisture, protein, fat, carbohydrate, crude fiber and ash contents as well as the energetic value, was expressed on a fresh weight (FW) basis (Table 2). According to ANOVA, all of the nutrient contents varied significantly ($P < 0.05$) among the plant samples. The moisture content was generally high in all shoot samples, especially in Enfidha (85.4 g/100 g FW) and Kerkennah (81.3 g/100 g FW) shoots. The protein levels were higher in Kerkennah (4.1) and Sijoumi (3.1 g/100 g FW) than in Enfidha (2.4 g/100 g FW) shoots. The highest rate of ash (5.2 g/100 g FW) was found in shoots from the Kerkennah site. Concerning crude fat and crude fiber, the greatest amounts were registered in Sijoumi shoots. Carbohydrates constituted the most abundant nutrient in all populations ($P < 0.01$); the highest content (19.1 g/100 g FW) was obtained in Sijoumi shoots, with a significantly lower rate (9.1 g/100 g FW) found in both Enfidha and Kerkennah shoots. The highest energetic value (94 kcal/100 g FW) was also recorded in Sijoumi shoots.

Mineral composition

As shown in Table 2, the results concerning the composition of the *B. macrocarpa* shoots in terms of macrominerals showed significant differences ($P < 0.05$) in the levels of Ca, Mg and Na. Na was the most abundant element found in *B. macrocarpa* shoots, especially at the Kerkennah site, with a concentration of about 1056 mg/100 g DW. Ca and Mg were found at their highest levels in shoots at the Sijoumi and Kerkennah sites. P was the least abundant macromineral in *B. macrocarpa* shoots (38–53 mg/100 g FW). The micro-mineral composition showed that Fe was the most abundant (4.6–9.6 mg/100 g FW), followed by Mn, Zn and Cu. Ni was detected in the smallest quantities, ranging from 0.06 to 0.09 mg/100 g FW (Table 2).

Functional properties

Functional properties of *B. macrocarpa* shoots were evaluated, including the total phenolic compounds (TPC), total flavonoid (TF) content and DPPH scavenging ability. Statistical analysis showed highly significant variation ($P < 0.01$) among shoot extracts. As shown in Table 4, TPC and TF recorded the highest values (of 39.01 mg GAE/g DW and 27.8 mg CE/g DW, respectively) in methanolic extracts of shoots from the Kerkennah site. Significantly lower values were found in shoots from the Enfidha (11.26 mg GAE/g DW; 5.86 mg CE/g DW) and Sijoumi (9.07 mg GAE/g DW; 4.61 mg CE/g DW) sites. Regarding DPPH scavenging activity, the results revealed that the antioxidant activity ($IC_{50} = 0.74$ mg/ml) of methanolic extracts of Kerkennah shoots was stronger than that of extracts from shoots from the Enfidha ($IC_{50} = 1.36$ mg/ml) and Sijoumi ($IC_{50} = 2.64$ mg/ml) sites.

Discussion

Soil properties

The analysis of physical and chemical parameters of the soil is of interest since those parameters strongly affect the growth and development of plants (Mehalaine and Chenchoumi 2020).

In our study, the analysis of physical parameters revealed different soil textures according to the USDA classification: Sijoumi soil is classified as loamy, Enfidha soil as silty clay loamy and Kerkennah soil as sandy. Regardless of their different textures, all soils displayed an alkaline pH and high electrical conductivity (12–32 dS/m), indicating very high salinity (Nguyen et al. 2020). In arid and semi-arid regions

such as our study area, the soils are generally alkaline and highly saline (Bello et al. 2021), which can be attributed to environmental factors like low precipitation, a high temperature and evaporation.

Regarding chemical properties, the results show that the soils contain different levels of total carbonate. According to Marschner (1995), these soils can be considered to be calcareous soils because they contain a certain amount of total carbonate. Total carbonate is an inorganic source of carbon that is mainly present in soils in arid and semi-arid areas in the form of calcium carbonate (CaCO₃) (Leogrande et al. 2021), which affects both the physical and chemical properties of the soil (Leogrande et al. 2021) as well as its fertility (by reducing the availability of nutrients; Matar et al. 1992).

The nutritional status of the sandy soil (at the Kerkennah site) was the poorest, especially in terms of organic carbon, organic matter and some minerals like Fe, Mn and Zn. The low fertility of sandy soil has also been reported in previous studies (Papafilippaki et al. 2015; Artikova et al. 2021) and has been attributed to the low nutrient retention capacity of such soils (Cambrollé et al. 2015). While the loamy soil exhibited relatively high fertility, especially in terms of organic matter and organic carbon, it is still poor (Dridi and Arfaoui 2017). In semi-arid regions, calcareous soils with high salinity and alkalinity are deficient in plant nutrients (Akhtar et al. 2019); in particular, organic matter (Aliat et al. 2016), organic carbon (Vimlesh and Giri 2011), organic nitrogen (Schröder 2014) and minerals (Akhtar et al. 2019; Wahba et al. 2019). Organic matter, which is the main reservoir of soil organic carbon and nitrogen (Hoffland et al. 2020), is reduced by high soil salinity (Qadir et al. 2000; Gonçalves Filho et al. 2019), leading to reductions in organic carbon and nitrogen. Moreover, the availability of minerals (P, K, Mg, Fe and Zn) is impaired in alkaline calcareous soils due to a nutritional imbalance between these minerals and calcium (Wahba et al. 2019).

Plant analysis

The analysis of the nutritional value of wild plants is crucial to demonstrate their edibility for humans. In this study, we assessed the nutritional properties of *Beta macrocarpa* shoots collected from three different sites (Sijoumi, Enfidha and Kerkennah) through their proximate and mineral compositions.

Proximate composition analysis showed a high moisture content in all *B. macrocarpa* shoots; the moisture content exceeded those found in *Beta maritima* (Tan et al. 2017), some leafy vegetables (Slavin and Lloyd 2012), and even halophytes such as *Arthrocnemum indicum*, *Halocnemum strobilaceum* and *Suaeda fruticosa* (Maatallah-Zaier et al. 2020). The low moisture content (73.6 g/100 g FW) recorded in Sijoumi shoots could be attributed to the high level of soil

Table 3 Percentages of the recommended dietary allowances (RDAs) of various nutrients covered by *Beta macrocarpa* shoots collected from the Sijoumi, Enfidha and Kerkennah sites

	Sijoumi	Enfidha	Kerkennah
Protein	6.1	4.7	8.1
Fat	0.9	0.4	0.5
Carbohydrates	7.3	3.5	3.5
P	7.5	7.1	5.4
Mg	55.1	30.5	68.2
Ca	31.2	12.6	42.4
K	19.2	22.5	29.9
Na	35.7	26.3	44.0
Zn	9.4	5.5	6.6
Mn	114.1	61.5	36.6
Fe	68.4	36.5	32.5
Cu	21.9	17.0	21.7

salinity. A similar observation has been reported for *Limonium algarvense* leaves, for which the moisture content level decreases with increasing salinity (Rodriguez et al. 2020).

This parameter is crucial to the plant's ability to withstand severe climatic conditions, especially salinity and drought (Flowers and Colmer 2008; Hameed and Khan 2011). It allows the elevated salt concentration and the osmotic pressure in the tissues of salt-stressed plants to be reduced (Dajic 2006). It is also a very important parameter for food processing, storage and food quality (Okello et al. 2018). *B. macrocarpa* shoots also showed higher crude protein levels (2.4–4.1 g/100 g FW) than those reported in the literature for wild (Tan et al. 2017; Pinela et al. 2017) and cultivated (Baião et al. 2017) beets as well as for some leafy vegetables such as green lettuce, red lettuce and spinach (Slavin and Lloyd 2012; Colonna et al. 2016). Proteins constitute about 20% of the human body (Satter et al. 2016), and they are involved in the building of muscles, bones and skin and in the control of many body functions through hormone formation (Mau et al. 1999). The highest protein content was found in shoots growing in the poorest soil (the Kerkennah site). This finding corroborates a previous study which showed that the protein level in leaves of *Crithmum maritimum* increased as soil fertility decreased (Martins-Noguerol et al. 2023). Protein metabolism is affected by a low availability of minerals, by interactions between them (Sun et al. 2018), and/or by an impoverishment in nitrogen, which is the main component of amino acids (Schlüter et al. 2013). So, the higher protein level obtained in Kerkennah shoots could be linked to the low nitrogen and mineral contents of the soil.

B. macrocarpa shoots can cover between 4.7 to 8.1% of the recommended daily protein intake (Table 3) (Meyers et al. 2006) and can therefore be proposed as a good protein source. Kerkennah shoots also accumulated the highest

ash level (5.2 g/100 g FW). The ash content represents the total amount of minerals within the biomass (Satter et al. 2016). It is vital for determining the nutritive value of foods (Agboola and Adejumo 2013). According to our results, the shoots of *B. macrocarpa* accumulated ash at a higher level than reported for *B. maritima* (Tan et al. 2017) and could provide a valuable amount of minerals for human consumption. *B. macrocarpa* shoots were also richer in crude fiber than *B. maritima* (Tan et al. 2017) and *B. vulgaris* (Baião et al. 2017) shoots and some leafy vegetables (Slavin and Lloyd 2012), covering around 24% of the recommended daily intake.

B. macrocarpa constitutes the vital source of essential fatty acids and energy (Suh et al. 2015) and is involved in many important cell mechanisms (Satter et al. 2016). Its shoots, which provided less than 1% of the recommended daily intake of fat (Table 3), can be considered a low-fat food (Meyers et al. 2006).

Fiber plays a crucial role in preventing several human diseases, such as cardiovascular pathologies, gastrointestinal disorders and colon cancer (Fuller et al. 2016). Also, it controls cholesterol blood levels and glycemia (Hounsome and Hounsome 2011). According to the American Institute of Medicine, *B. macrocarpa* shoots constitute a potential source of fiber for nutrition and the food industry (Chau and Huang 2003).

Carbohydrates constitute the most abundant nutrient (9.1–19.1 g/100 g FW) found in shoots from all populations. These values are higher than those previously reported for other species belonging to the same genus, such as *Beta maritima* (Tan et al. 2017; Pinela et al. 2017) and *Beta vulgaris* (Baião et al. 2017), and can cover between 3.5 and 7.3% of the total daily recommended intake of carbohydrates (260 g/day for adults) (Table 3) (Meyers et al. 2006). Carbohydrates are the most abundant compounds in living plants and constitute the main source of energy production for our body's cells and the brain (El-Amier et al. 2022; Ebifa-Othieno et al. 2020). Also, they act as regulators and substrates for several specific biochemical processes (Bokov et al. 2017). The highest level was registered in shoots from the Sijoumi site, which could be related to the fact that those shoots also had the highest fiber and carbohydrate contents (El-Amier et al. 2022) and therefore the highest energetic value. Energy is essential for many functions of the body, such as respiration and protein synthesis (Ebifa-Othieno et al. 2020).

The mineral compositions of edible vegetables represent one of the most important aspects that influence their use in human nutrition. Minerals (macro- and microminerals) are essential for the proper functioning of the human body (Quintaes and Diez-Garcia 2015). A deficiency in these minerals is considered a form of malnutrition in many developing countries (Mohanty et al. 2016) and is also considered a health problem, since it leads to many chronic and

degenerative disorders (Mzoughi et al. 2019). The results for the macrominerals, which are needed in relatively large amounts to achieve some physiological functions in our body (Mohanty et al. 2016), reveal that Na is the most abundant element in *B. macrocarpa* shoots. The Na levels greatly surpassed those found in wild beet *B. maritima* (Tan et al. 2017; Pinela et al. 2017) and some varieties of Swiss chard (*B. vulgaris* subsp. *Cicla*) (Pokluda and Kuben 2002). According to Hamouda et al. (2016), *B. macrocarpa* plants which were exposed to salinity stress accumulated high amounts of Na in their tissues. The same observations were also reported for some halophytes such as *Sarcocornia perennis*, *Salicornia ramosissima*, *Arthrocnemum macrostachyum* (Barreira et al. 2017), *Mesembryanthemum nodiflorum*, *Suaeda maritima* and *Sarcocornia fruticosa* (Castañeda-Loaizaa et al. 2020). So, it seems that *B. macrocarpa* plants can behave like halophytes by accumulating Na in their tissues without showing any symptoms of toxicity (Papafilippaki et al. 2015). Na is a metabolically toxic ion that is used by halophytes to control cell osmotic adjustment (Flowers 1985). Concerning human consumption, Na is an essential mineral that should be consumed in moderate amounts to avoid cardiovascular diseases (Kotchen et al. 2013). According to the World Health Organization (WHO), the daily intake of sodium should be less than 2000 mg (Castañeda-Loaizaa et al. 2020). With regard to the edibility of *B. macrocarpa*, the shoots harvested from the Sijoumi, Enfidha and Kerkennah sites would, respectively, provide 632, 1056 and 856 mg/100 g FW of Na (Table 2) and cover about 35, 26 and 44% of the daily recommended intake (Table 3) (Meyers et al. 2006).

Regarding potassium, *B. macrocarpa* can be considered a good source of this nutrient since it can provide appreciable amounts (385–598 mg/100 g FW) (Table 2) and can cover from 19 to 30% of the daily recommended intake of K (Table 3) (Meyers et al. 2006). So, *B. macrocarpa* can be regarded as a good source of K if consumed in moderation.

Ca is also accumulated to higher levels than in *B. maritima* (Tan et al. 2017; Pinela et al. 2017). This element is implicated in the salt tolerance process in salt-resistant plants as well as in ion uptake and transport (Yamanouchi et al. 1997). Its consumption is crucial for many body functions, like the blood clotting process, nervous system control, and bone and teeth formation (Mohanty et al. 2016). According to the results illustrated in Table 3, *B. macrocarpa* shoots can be considered a valuable source of Ca, as they could cover from 12 to 40% of our daily intake (Meyers et al. 2006). The elevated content of Ca in Kerkennah shoots is probably associated with the excess of Na in the soil; the high level of Ca could be used in a defence mechanism exerted by the plant to re-establish homeostatic conditions in the presence of high Na accumulation (Tipirdamaz et al. 2006). Mg is an essential component of bone and cartilage and is a cofactor for many enzymes involved in energy

metabolism and the synthesis of protein, RNA and DNA (Mohanty et al. 2016). According to the data obtained, *B. macrocarpa* can supply significant quantities of Mg: enough to cover almost 70% of the recommended daily intake (Meyers et al. 2006). The least abundant macromineral in *B. macrocarpa* shoots was P; the shoots can supply only 7% of the body's daily requirements for this nutrient (Meyers et al. 2006). In fact, the availability of P to the plant for uptake and utilization is impaired in alkaline and calcareous soils due to the formation of poorly soluble calcium phosphate minerals (Hopkins and Ellsworth 2005).

Microminerals (Fe, Mn, Cu and Zn), which are required in trace amounts, are also important for normal functioning of the body and play a major role in redox processes (Mzoughi et al. 2019). Fe was the most abundant element among the microminerals and was found at higher concentrations than in *B. maritima* (Pinela et al. 2017). This could be linked to its abundance in the soil. Approximately 70% of our daily recommended intake of this element could be provided by *B. macrocarpa* shoots (Table 3) (Meyers et al. 2006). Fe is a very important element for body functioning, and a deficiency of Fe can cause several diseases, like anemia (Quintaes and Diez-Garcia 2015) as well as impaired physical and cognitive development and an increased risk of morbidity in children (WHO 2005). Mn is also an important element in several body functions, like fat and carbohydrate metabolism, brain and nerve function, calcium absorption (Mohanty et al. 2016), and blood sugar regulation (Gupta and Gupta 2014). Given the elevated Mn content in *B. macrocarpa* shoots—especially in shoots at the Sijoumi site (2.3 mg/100 g FW), which could cover more than the daily requirement of Mn (114%) (Table 3) (Meyers et al. 2006)—the consumption of this species in small quantities would meet the adequate daily intake level of Mn. Zn and Cu are also very important minerals; they are required for Fe utilization, glucose metabolism, hemoglobin synthesis (Celik and Oehlenschlaeger 2004) and cell division (Gupta and Gupta 2014). In *B. macrocarpa* shoots, these two minerals were found at similar levels to those in *B. maritima* (Tan et al. 2017; Pinela et al. 2017). Similarly to some wild edible plants, small amounts of Ni were detected in *B. macrocarpa* shoots (Shad et al. 2013; Barreira et al. 2017). In alkaline calcareous soils, the interactions of nutrients and their mobilization within the plant play a decisive role in nutrient acquisition (Akhtar et al. 2019). The uptake and use of minerals (P, K, Mg, Fe and Zn) by plants are generally altered due to a nutritional imbalance between these minerals and calcium (Wahba et al. 2019).

Functional properties of *B. macrocarpa* shoots were assessed, including the total phenolic compounds (TPC), total flavonoid (TF) content and DPPH scavenging ability.

Table 4 Phenolic contents and DPPH antioxidant activity in methanolic extracts of *B. macrocarpa* shoots collected from the Sijoumi, Enfidha and Kerkennah sites

	Sijoumi	Enfidha	Kerkennah
TPC (mg GAE/g DW)	9.07 ± 1.80 ^b	11.26 ± 1.90 ^b	39.01 ± 4.18 ^a
TF (mg CE/g DW)	4.61 ± 2.36 ^b	5.86 ± 1.26 ^a	27.80 ± 3.32 ^a
DPPH (IC ₅₀ , mg/ml)	2.64 ± 2.22	1.36 ± 0.68	0.74 ± 0.34

Values are means of three replicates ± standard deviation. Values with different superscripts (a, b) are significantly different at $P < 0.05$. TPC is expressed in mg gallic acid equivalent per g dry weight; TFC is expressed in mg catechin equivalent per g dry weight. DPPH is expressed in mg per mL of the extract dose required to cause a 50% decrease in the absorbance at 517 nm

Phenolic compounds are secondary metabolites that are synthesized through the shikimic acid and phenylpropanoid pathways in most plant tissues (Table 4). They are important plant constituents which provide many health benefits (de la Rosa et al. 2019), such as antioxidant, anti-inflammatory, anti-tumor and antimicrobial properties (Vermerris and Nicholson 2008). Many previous studies have shown that the consumption of a phenolic- and flavonoid-rich natural diet is linked to improved health and a decreased incidence of neurodegenerative and cardiovascular diseases, cancer and diabetes (Aryal et al. 2019). Moreover, phenolic compounds are known to play a crucial role in the adaptation of plants to their environment (Chowdhary et al. 2021). So, the accumulation of phenolic compounds in methanolic extracts of *B. macrocarpa* shoots, especially those from the Kerkennah site, could be a response to the stressful conditions to which they were subjected. These plants accumulated more phenolic compounds to prevent oxidative stress and the production of radical oxygen species (Bose et al. 2014), which were generated by the salinity of the soil and the arid climate of the Kerkennah site. The antioxidant activity of the shoot extracts was assessed through the DPPH assay, which is the most widely used technique to evaluate radical scavenging potency (Aryal et al. 2019). Our results showed higher antioxidant activity in Kerkennah shoots, which was probably correlated to a high accumulation of phenolic compounds.

The obtained results indicate that *Beta macrocarpa* shoots constitute a potential source of valuable nutrients (proteins, fiber, carbohydrates and minerals) and phenolic compounds with high scavenging potency. Thus, their consumption could provide health benefits and improve modern diets (Romojaro et al. 2013). Due to its tolerance to high salinity, low nutrient availability and an arid climate on the one hand and its nutritional value and functional properties on the other, *Beta macrocarpa* could be considered as an alternative source of high-quality food as a part of a sustainable agriculture in marginal environments.

Conclusions

The results show that Tunisian *Beta macrocarpa*, which is one of the most important halophytes and CWR species (especially in the Mediterranean Basin, where it is ranked as an endangered (EN) taxon), deserves to be valorized for its nutritional value and to be preserved ex situ in a gene bank. Chemical analysis showed that *B. macrocarpa* has high nutritional value due to its proximate composition and its mineral contents. The proximate composition of *B. macrocarpa* shoots revealed substantial protein, a low fat content, fiber and a high carbohydrate content, which confirm the possibility of using this vegetable in salads (sautéed or cooked) and/or in feed, as it has high levels of energy-storage compounds. In addition, *B. macrocarpa* shoots accumulated high mineral element contents—essentially Na, K, Ca, Mg and Fe. Na and K play an important role as they are components of active osmotic adjustment, which confirms that the species is perfectly salt tolerant. The Tunisian *B. macrocarpa* is a halophyte species which plays a considerable role in the Mediterranean diet, as it can be used universally in different traditional dishes because of its high nutritional value and mineral content. The large-fruited beet presents high functional properties as it accumulates secondary metabolites (polyphenols and flavonoids) that are synthesized during stress conditions, such as salt stress. These metabolites act as antioxidants, participating in plant growth, reproduction and resistance to pathogens. Other nutritional aspects of this species, such as its vitamin composition, anti-nutritional factors and compounds that are potentially toxic to humans or animals, should be further investigated.

According to our results, the consumption of leaves from *B. macrocarpa* can contribute to a balanced diet and may be used in modern diets, as a dietary supplement or as a functional ingredient in the design of novel organic food products. It can be used as food and/or as a feed plant as it is rich in important components with high nutritional value. It can be grown and cultivated according to the organic farming system, which is based on maintaining biodiversity and ecological services and principles. *B. macrocarpa* can be cultivated in marginal areas in order to limit fodder deficits and to improve the pastoral value of these regions while conserving their natural resources, and therefore it could be selected as a useful species in many restoration environmental projects in the Mediterranean area, such as REACT4MED (<https://react4med.eu/>) and EcoplantMed (<http://www.ecoplantmed.eu/project/>).

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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