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Studies on Chemical Constituents and Bioactivity of *Rosa micrantha*: An Alternative Antioxidants Source for Food, Pharmaceutical, or Cosmetic Applications

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Rose species have long been used for food and medicinal purposes. *Rosa micrantha* is one of the rose species that grow feral in the northeastern Portuguese region so-called Nordeste Transmontano. For the first time, chemical composition and bioactivity of their petals, fertilized flowers, unripe, ripening, and overripe hips were evaluated in order to valorize them as sources of important phytochemicals. Chemical characterization included determination of proteins, fats, ash, and carbohydrates, particularly sugars, by HPLC-RI, fatty acids by GC-FID, tocopherols by HPLC-fluorescence, and phenolics, flavonoids, carotenoids, and ascorbic acid by spectrophotometric techniques. Bioactivity was evaluated through screening of antioxidant properties: radical scavenging effects, reducing power, and inhibition of lipid peroxidation. Ripening and overripe hips showed high nutritional value including proteins, carbohydrates, omega-3 and omega-6 fatty acids, energy, sugars, particularly the reducing sugars fructose and glucose, and ascorbic acid (>693 mg/100 g). Fertilized flowers and petals revealed the highest antioxidant activity (EC_{50} > 152 μg/mL) and phenolics, flavonoids, and tocopherols contents (>35 mg/100 g). Furthermore, petals, ripening, and overripe hips are important sources of carotenoid pigments (>64 mg/100 g). Because of the diversity and abundance of antioxidants found in this species, some food, cosmetic, and pharmaceutical applications could be explored.

KEYWORDS: ***Rosa micrantha*; hips; flowers; bioactive compounds; applications; Portuguese ethnobotany**

INTRODUCTION

Reactive oxygen (ROS) and nitrogen (RNS) species production inevitably occurs during normal cell metabolism both in animals and plants. An excess of those species lead to oxidative and nitrosative stresses, resulting in damage for some cellular molecules such as DNA, proteins, and lipids (*1*, *2*). The reactions involved in the generation of ROS and RNS in biological systems by multiple processes seem to be a key process in food spoilage, in aging, and in a number of human diseases such as atherosclerosis, reperfusion injury, cataractogenesis, rheumatoid arthritis, inflammatory disorders, and cancer (*1*, *2*). Therefore, natural antioxidants have become a safety option to avoid biological oxidations involved in cellular damage and deterioration of food quality (*3*).

Members of the Rosaceae family have long been used for food and medicinal purposes. The physiological functions of Rosaceae fruits may be partly attributed to their abundance of compounds such as phenolics, β-carotene, lycopene, ascorbic acid, tocopherol, bioflavonoids, fruit acids, tannins, pectin, sugars, organic acids, amino acids, and essential fatty acids (*4*). They are traditionally used as a dietary supplement and/or medicine in many cultures (*5*,*6*).

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As a popular vitamin C enriched herbal dietary supplement, rose hips are used in a lot of food preparations such as tea, jelly, jam, and alcoholic beverages after fermentation (*5*, *6*). As an herbal remedy, rose hips are used for the treatment of various diseases including cold, flu, inflammation, and chronic pain, for skin care, and for their antiulcer properties (*5*, *7*, *8*). The oil extracted from the seeds is included in many cosmetic preparations for its high content of oleic, linolenic, and linoleic fatty acids (*9*−*14*).

Some literature can be found about composition and characterization of chemicals occurring in oilseed and fruits of some *Rosa* spp. such as *R. rubiginosa* (*4*, *9*, *14*, *15*) and *R. canina* (*16*), but not of *Rosa micrantha*.

Native to Europe and Western Asia, in Portugal, *Rosa micrantha* Borrer ex Sm. is a dense deciduous shrub of the edges of oak forests, sharing this habitat with other wild roses from the *Rosa canina* group and other Rosaceae species such as *Crataegus monogyna* and *Prunus spinosa*. Leaves and peduncles are sparsely to densely covered with glandular hairs which have an apple-like fragrance. The fleshy edible fruit is a globose to oblong reddish hip which begins to form in spring and ripen in late summer through autumn (*17*). Several European ethnobotanical surveys highlighted the importance of wild roses in folk medicine and personal care, as well as ingredient in traditional recipes (*18*−*20*). However, none of them refers specifically to *R. micrantha*, which is a species highly

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praised in the northeastern Portuguese region due to its characteristic apple scent, even if only skillful gathers/users are able to distinguish *R. micrantha* from the other roses of the section (*21*). Local key informants categorized and described the multiple use of this particular species as medicinal and veterinary, edible, ornamental, and ritual while they showed the plants they have obtained by cuttings brought from the woods (*21*). Beneficial health and cosmetic properties of its flowers and hips have been transmitted and tapped by natives for centuries (*21*), but there are no reports on chemical characterization of this species that support the empirical/folk uses.

The aim of the present work is to find quantitative data on the chemical constituents and bioactivity of *R. micrantha* that support the folk applications and open possibilities of food, cosmetic, and pharmaceutical applications, similarly to other rose species such as *R. rubiginosa* (*5*, *7*, *8*, *10*−*13*, *18*−*20*).

MATERIALS AND METHODS

Samples. Samples for analysis were gathered and prepared according to the main medicinal or edible practices and uses described by Portuguese informants from the northeastern part of the country (Bragança, Portugal). Five different samples were collected in sequence, during 2009 spring, summer, and autumn, synchronized with the species' development of flowers and fruits. Two of the samples were petals removed from floral buds and fertilized flowers (flowers after anthesis, anthers already opened, stamens becoming dry). The remaining three were fruits at various stages of ripeness: unripe hips, corresponding to flower senescence and stand out of the immature green fruits; ripening hips, that is, orange−reddish hard fruits in late summer; and overripe hips, that is, fleshy and soft dark-red fruits in late autumn (**Figure 1**).

Morphological key characters from the Flora Iberica (*17*) were used for plant identification. Voucher specimens were deposited in the herbarium of the Escola Superior Agrária de Bragança (BRESA). Each sample was lyophilized in an Ly-8-FM-ULE equipment (Snijders, Holland) and kept in the best conditions for subsequent use.

Standards and Reagents. Acetonitrile 99.9%, *n*-hexane 95%, and ethyl acetate 99.8% were of HPLC grade (Lab-Scan, Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO), as were also other individual fatty acid isomers, ascorbic acid, tocopherols, and sugars standards, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, and (+)-catechin. Racemic tocol, 50 mg/mL, was purchased from Matreya (Chalfont, PA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (Pure Water Systems, Brea, CA).

Chemical Constituents. *Macronutrients*. The samples were analyzed for chemical composition (moisture, protein, fat, carbohydrates, and ash) using the AOAC procedures (*22*). Protein content ($N \times 6.25$) of the samples was estimated by the macro-Kjeldahl method; fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Carbohydrates were calculated by difference: carbohydrates = 100 − (g protein + g fat + g ash). Reducing sugars were determined by DNS (dinitrosalicylic acid) method. Energy was calculated according to the following equation: energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g lipid).

Fatty Acids. Fatty acids were determined by gas chromatography with flame ionization detection (GC-FID) as described previously by the authors (*16*). The equipment was a DANI model GC 1000 with a split/splitless injector and a FID. The column used was a 30 m × 0.32 mm i.d., 0.25 μm, 50% cyanopropyl-methyl-50% phenylmethylpolysiloxane (Macherey-Nagel, Düren, Germany). The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 10 °C/min ramp to 240 °C and held for 11 min. The carrier gas (hydrogen) flow rate was 4.0 mL/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW DataApex 1.7 software and expressed in relative percentage of each fatty acid.

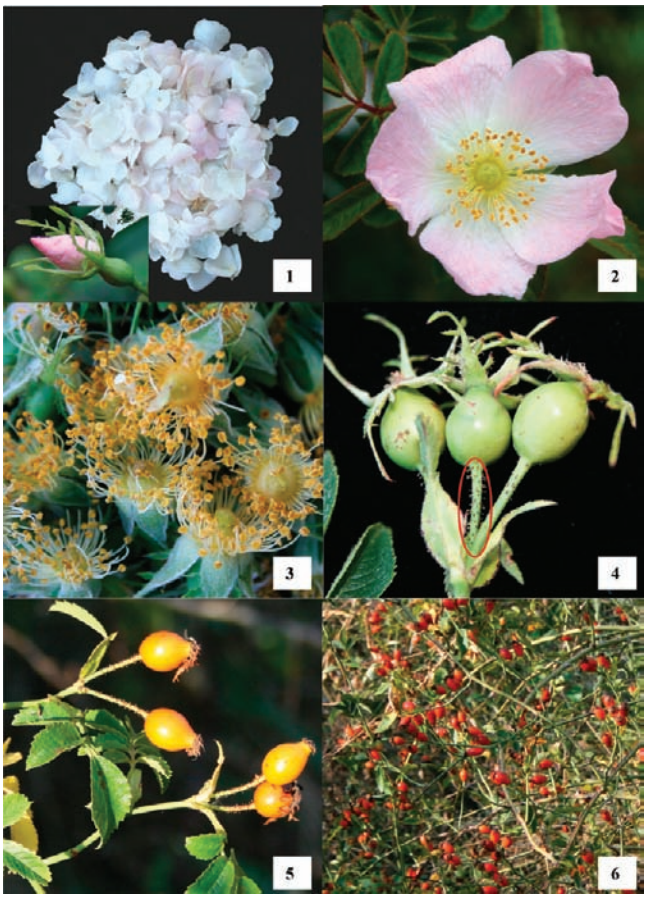

Figure 1. *Rosa micrantha* Borrer ex Sm. Different samples used for analysis according informants criteria of use. (**1**) Petals removed from floral buds (detail of bud); (**2**) flower after anthesis (fertilized flower); (**3**) detail of hips from fertilized flowers; (**4**) unripe hips and detail of glandular hairs; (**5**) ripening hips; (**6**) plant presenting overripe hips in late autumn.

Sugars. Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) as previously described by the authors (*16*), using melezitose as internal standard. The equipment consisted of an integrated system with a Smartline 1000 pump (Knauer, Berlin, Germany), a Smartline manager 5000 degasser, an AS-2057 autosampler (Jasco, Easton, MD), and a Smartline 2300 RI detector. Data were analyzed using Clarity DataApex 2.4 Software. The column used was a 250 mm ×4.6 mm i.d., 5 μm, Eurospher 100-5 NH_2 with a 5 mm ×4 mm i.d. guard column of the same material (Knauer, Berlin, Germany), operating at 30 °C in a 7971 R Grace oven. The mobile phase was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1 mL/min. Sugar identification was made by comparing the relative retention times of sample peaks with standards. Quantification was made by internal normalization of the chromatographic peak area, and the results are expressed in g per 100 g of dry weight.

Tocopherols. Tocopherols content was determined following a procedure previously optimized and described by the authors (*23*), using tocol as internal standard. The HPLC system described above was connected to a FP-2020 fluorescence detector (Jasco, Easton, MD) programmed for excitation at 290 nm and emission at 330 nm. The column used was a normal-phase 250 mm ×4.6 mm i.d., 5 μm, polyamide II, with a 10 mm × 4 mm i.d. guard column of the same material (YMC Waters, Dinslaken, Germany), operating at 30 °C. The mobile phase used was a mixture of *n*-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method. Tocopherol contents in the samples are expressed in mg per 100 g of dry weight.

Table 1. Composition in Moisture and Nutrients, and Energetic Value of *R. micrantha* (Mean ± SD; $n = 9$)[a]

	petals	fertilized flowers	unripe hips	rRipening hips	overripe hips
moisture (g/100 g fw)	71.62 ± 6.89 a	67.97 ± 5.10 b	51.19 ± 3.12 c	44.54 ± 1.22 d	37.81 ± 1.19 e
ash (g/100 g dw)	4.22 ± 0.01 b	6.84 ± 0.08 a	4.28 ± 0.21 b	3.16 ± 0.21 c	4.19 ± 0.73 b
proteins (g/100 g dw)	4.32 ± 0.06 a	3.48 ± 0.17 b	4.21 ± 0.17 a	4.18 ± 0.06 a	4.12 ± 0.02 a
fat (g/100 g dw)	1.31 ± 0.04 b	0.92 ± 0.07 c	0.37 ± 0.09 e	0.68 ± 0.11 d	1.43 ± 0.18 a
carbohydrates (g/100 g dw)	90.15 ± 0.08 c	88.76 ± 0.23 d	91.14 ± 0.19 b	91.98 ± 0.26 a	90.16 ± 0.55 c
reducing sugars (g/100 g dw)	9.63 ± 0.00 b	5.45 ± 0.49 d	1.06 ± 0.01 e	7.41 ± 0.01 c	17.85 ± 0.90 a
energy (kcal/100 g dw)	389.71 ± 0.10 a	377.22 ± 0.01 c	384.75 ± 0.27 b	390.80 ± 0.20 a	390.81 ± 1.83 a

[a] fw: fresh weight; dw: dry weight. In each row, different letters mean significant differences ($p < 0.05$).

Ascorbic Acid. Ascorbic acid was determined following a procedure previously described by the authors (*23*) with 2,6-dichloroindophenol, and measuring the absorbance at 515 nm in a 200−2004 spectrophotometer (Analytikjena, Jena, Germany). Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.006−0.1 mg/mL), and the results were expressed as mg of ascorbic acid per g of dry weight.

Carotenoids. β-Carotene and lycopene were determined following a procedure previously described by the authors (*23*), measuring the absorbance at 453, 505, 645, and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 mL) = $-0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453}$; β-carotene (mg/100 mL) = $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$. The results were expressed as mg of carotenoid per g of dry weight.

Phenolics. A fine dried powder (20 mesh; ∼1 g) was extracted by stirring with 50 mL of methanol at 25 °C at 150 rpm for 12 h and filtered through Whatman no. 4 paper. The residue was then extracted with one additional 50 mL portion of methanol. The combined methanolic extracts were evaporated at 35 °C under reduced pressure, redissolved in methanol at a concentration of 10 mg/mL, and stored at 4 °C for further use.

For total phenolics estimation, it was followed a spectrophotometric assay previously described by the authors (*23*), with measurement of absorbance at 765 nm. Gallic acid was used to calculate the standard curve (0.05−0.8 mM), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

For total flavonoids content determination, it was followed a spectrophotometric assay previously described by the authors (*23*), with measurement of absorbance at 510 nm. (+)-Catechin was used to calculate the standard curve (0.0156−1.0 mM), and the results were expressed as mg of (+)-catechin equivalents (CE) per g of extract.

Antioxidant Activity Assays. In vitro assays already described by the authors in previous studies (*16*, *23*) were applied to evaluate the antioxidant activity of all the samples. Different concentrations of the extracts (1.00−0.03 mg/mL) were used to find EC_{50} values.

DPPH Radical-Scavenging Activity. This methodology was performed using an ELX800 microplate reader (BioTek Instruments, Inc., Winooski, VT). The reaction mixture in each one of the 96 wells consisted of one of the different concentrations of the extracts (30 μL) and aqueous methanolic solution (80:20 v/v, 270 μL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was kept for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. Trolox was used as standard.

Reducing Power. This methodology was performed using the microplate reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48 wells, as were also deionized water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 690 nm against extract concentration. Trolox was used as standard.

Inhibition of β-Carotene Bleaching. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two mL of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40 °C under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. A blank, devoid of β-carotene, was prepared for background subtraction. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2 h of assay/initial β-carotene content) × 100. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration. Trolox was used as standard.

Inhibition of Lipid Peroxidation Using Thiobarbituric Acid Reactive Substances (*TBARS*). Brains were obtained from pig (*Sus scrofa*) of body weight ∼150 kg, dissected, and homogenized with a Polytron in ice-cold Tris−HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate, which was centrifuged at 3000*g* for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the different concentrations of the extracts (0.2 mL) in the presence of $FeSO_4$ (10 μM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37 °C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)−TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = $[(A - B)/A] \times 100\%$, where *A* and *B* were the absorbance of the control and the compound solution, respectively. The extract concentration providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the graph of TBARS inhibition percentage against extract concentration. Trolox was used as standard.

Statistical Analysis. For each one of the samples, the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$. This treatment was carried out using SPSS version 16.0 program.

RESULTS

Chemical Constituents. The results of the nutrients composition and estimated energetic value (expressed on dry weight basis) obtained for the different parts of *R. micrantha* are shown in **Table 1**. Petals revealed the highest moisture content (71.62 g/100 g), while overripe fruits showed the lowest content (37.81 g/100 g). Ash was more abundant in fertilized flowers (6.84 g/100 g) and less abundant in ripening hips (3.16 g/100 g), and could include elements such as P, K, Ca, Mg, Fe, Cu, Mn, and Zn as it was described for different rose fruits (*4*). Protein was found in low levels and varied between 3.48 g/100 g in fertilized flowers and 4.32 g/100 g in petals. All the studied parts of *R. micrantha* revealed higher levels of protein than fruits from *R. canina* (*4*, *16*), *R. dumalis* subsp. *boissieri*, *R. dumalis* subsp. *antalyensis*, *R. villosa*, *R. pisiformis*, and *R. pulverulenta* (*4*), which presented values

Table 2. Composition in Fatty Acids of *R. micrantha* (Mean ± SD; $n = 9$)[a]

	petals	fertilized flowers	unripe hips	ripening hips	overripe hips
C6:0	0.13 ± 0.02	0.32 ± 0.03	0.05 ± 0.00	0.05 ± 0.00	0.07 ± 0.00
C8:0	0.44 ± 0.10	0.61 ± 0.03	0.06 ± 0.00	0.07 ± 0.00	0.04 ± 0.00
C10:0	0.33 ± 0.07	0.42 ± 0.04	0.13 ± 0.01	0.19 ± 0.00	0.05 ± 0.00
C12:0	0.87 ± 0.06	1.80 ± 0.05	1.01 ± 0.01	0.20 ± 0.00	0.29 ± 0.09
C14:0	1.48 ± 0.25	2.67 ± 0.21	1.06 ± 0.01	1.18 ± 0.03	0.23 ± 0.06
C14:1	nd	0.11 ± 0.02	nd	0.20 ± 0.02	0.07 ± 0.00
C15:0	0.22 ± 0.04	0.28 ± 0.00	0.32 ± 0.02	0.16 ± 0.01	0.09 ± 0.02
C15:1	1.00 ± 0.04	1.05 ± 0.05	0.07 ± 0.00	nd	nd
C16:0	11.31 ± 0.02	14.37 ± 0.31	7.43 ± 0.09	4.54 ± 0.56	5.24 ± 0.35
C16:1	nd	nd	0.04 ± 0.00	0.36 ± 0.02	0.23 ± 0.03
C17:0	0.67 ± 0.04	0.73 ± 0.00	0.55 ± 0.06	0.47 ± 0.01	0.16 ± 0.00
C18:0	6.62 ± 0.15	7.25 ± 0.03	4.51 ± 0.14	3.32 ± 0.79	2.29 ± 0.06
C18:1n9c	1.80 ± 0.11	3.45 ± 0.49	7.27 ± 0.11	10.22 ± 0.07	12.74 ± 0.77
C18:2n6c	21.18 ± 0.70	18.98 ± 0.90	40.39 ± 0.26	33.79 ± 0.12	44.32 ± 0.04
C18:3n3	32.33 ± 0.18	28.42 ± 0.80	26.25 ± 0.12	26.99 ± 0.25	29.53 ± 1.34
C20:0	3.73 ± 0.34	3.89 ± 0.55	1.30 ± 0.12	1.54 ± 0.12	0.99 ± 0.02
C20:1c	0.60 ± 0.04	0.45 ± 0.04	0.62 ± 0.07	0.62 ± 0.00	0.33 ± 0.00
C20:3n3 + C21:0	0.29 ± 0.01	0.16 ± 0.04	0.19 ± 0.00	0.66 ± 0.01	0.32 ± 0.00
C22:0	4.39 ± 0.13	5.16 ± 0.69	1.02 ± 0.03	0.69 ± 0.02	0.33 ± 0.00
C23:0	9.27 ± 0.84	7.76 ± 0.03	1.37 ± 0.03	1.70 ± 0.09	1.06 ± 0.02
C22:6n3	nd	nd	5.52 ± 0.49	10.14 ± 0.03	1.27 ± 0.06
C24:0	3.36 ± 0.07	2.13 ± 0.60	0.83 ± 0.01	2.43 ± 0.36	0.34 ± 0.01 e
total SFA	42.81 ± 0.33 b	47.39 ± 0.50 a	19.65 ± 0.06 c	17.03 ± 0.16 d	11.19 ± 0.52 a
total MUFA	3.39 ± 0.19 e	5.05 ± 0.43 d	8.01 ± 0.17 c	11.40 ± 0.17 b	13.37 ± 0.80 a
total PUFA	53.80 ± 0.52 c	47.56 ± 0.06 d	72.34 ± 0.12 b	71.57 ± 0.33 b	75.44 ± 1.32 a
PUFA/SFA	1.26 ± 0.02 d	1.00 ± 0.01 d	3.68 ± 0.00 c	4.20 ± 0.06 b	6.75 ± 0.43 a
n-6/n-3	0.66 ± 0.03 d	0.67 ± 0.05 d	1.27 ± 0.02 b	0.91 ± 0.00 c	1.44 ± 0.06 a

[a] nd: not detected. In each row, different letters mean significant differences ($p < 0.05$).

between 0.98 and 2.72 g/100 g. Fat was the macronutrient less abundant being lower than 1.5 g/100 g; ripening hips of *R. micrantha* (**Table 1**) showed similar fat levels to fruits from *R. canina* (*16*). Carbohydrates, calculated by difference, were the most abundant macronutrients and were higher than 88.7 g/100 g. Reducing sugars are only a small part of carbohydrates due to the abundant presence of polysaccharides such as starch and cellulose. The highest energetic values were obtained in petals, ripening and overripe hips (**Table 1**) that presented statistically ($p < 0.05$) similar values. The macronutrients profile of *R. canina* fruits described by us in a previous study (*16*) was similar to ripening hips of *R. micrantha*, indicating that the fruits used were probably in the same stage of maturity. Nevertheless, differences in chemical properties of fruits having about the same size might occur due to environmental conditions in conjunction with the analytical methods used (*24*).

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and the ratios of PUFA/SFA and n-6/n-3 of the studied parts of *R. micrantha* are shown in **Table 2**. The major fatty acids found were α-linolenic acid (C18:3n3) and linoleic acid (C18:2n6), contributing to the prevalence of PUFA. Petals and fertilized flowers also revealed palmitic acid (C16:0) as a main fatty acid, which increase the SFA levels found in these samples. Otherwise, oleic acid (C18:1n9) was abundant for ripening and overripe hips, contributing to the increase of their MUFA contents. Docosahexaenoic acid (C22:6n3) was characteristic of hips samples. Twenty-two fatty acids were identified and quantified, and the chromatogram obtained for overripe hips is shown as an example (**Figure 2**). The UFA oleic, linoleic, and linolenic acids have also been reported as main fatty acids in *R. rubiginosa* (*9*, *13*, *14*), *R. canina* (*4*, *16*), *R. dumalis* subsp. *boissieri*, *R. dumalis* subsp. *antalyensis*, *R. villosa*, *R. pisiformis*, and *R. pulverulenta* (*4*).

In relation to sugar composition (**Table 3**), petals, fertilized flowers, and overripe hips gave fructose as the main sugar, while sucrose predominated in unripe and ripening hips. Overripe hips revealed the highest total sugars content (22.21 g/100 g), with the highest levels of fructose (10.30 g/100 g) and glucose (9.98 g/100 g), which is in agreement with their sweet taste. In fact, fructose is known to be the sweetest of all naturally occurring carbohydrates (*25*). The chromatogram obtained for this sample is presented in **Figure 3**. Otherwise, unripe hips showed the lowest levels in total sugars (2.53 g/100 g). Total sugars determined by HPLC-RI (**Table 3**) were higher than reducing sugars obtained by DNS method (**Table 1**) due to the contribution of nonreducing sugars such as sucrose and trehalose.

Vitamins (tocopherols and ascorbic acid) and carotenoids (β-carotene and lycopene) contents in *R. micrantha* are given in **Table 4**. The values obtained in the analysis of the different parts point to the existence of differences in what concerns tocopherols composition. α-Tocopherol was the major compound in all the samples (as it can be seen in **Figure 4**), β-tocopherol was not detected in unripe and ripening hips, and in this last sample, δ-tocopherol was also not detected. Fertilized flowers presented the highest content of tocopherols (42.08 mg/100 g of dry weight), while hips revealed the lowest contents, and no statistical differences ($p < 0.05$) were observed for the different stages of maturity. In a previous study (*16*), we reported the presence of tocopherols in *R. canina* fruits (8.33 mg/100 g), this quantity being lower than the concentration found in *R. micrantha* (19.64 mg/100 g). Other authors published tocopherols determination in *R. rubiginosa* shell extracts, but the values were expressed in the extract mass and not in a dry weight basis, which cannot be comparable; furthermore, they only describe the presence of α-tocopherol (*26*).

Ascorbic acid was the most abundant vitamin in all the parts of *R. micrantha*; ripening hips gave the highest levels (943.89 mg/100 g of dry weight; **Table 4**). Ercisli (*4*) reported the quantification of

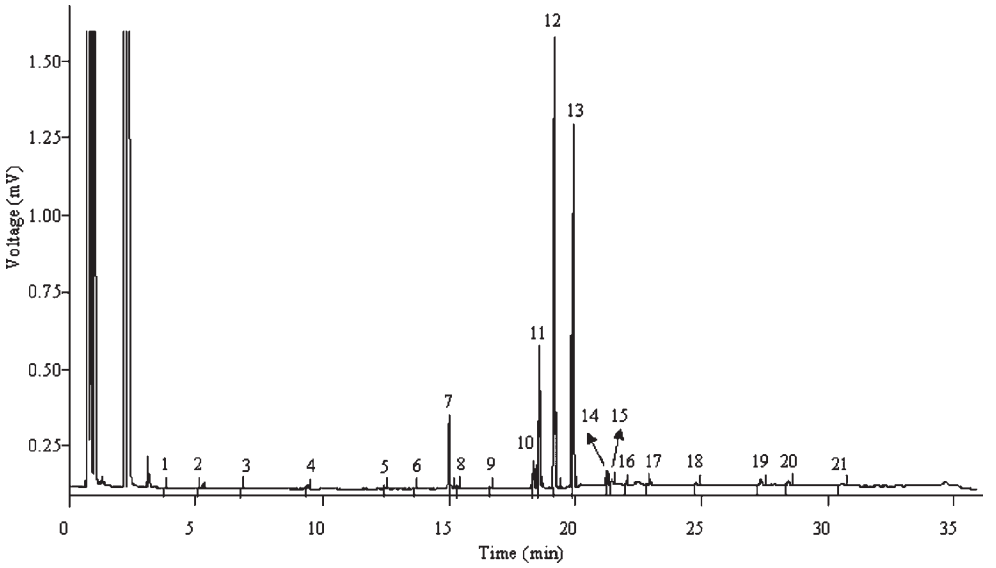

Figure 2. Individual fatty acids chromatogram of overripe hips of *R. micrantha*. (**1**) Caproic acid (C6:0); (**2**) caprylic acid (C8:0); (**3**) capric acid (C10:0); (**4**) lauric acid (C12:0); (**5**) myristic acid (C14:0); (**6**) pentadecanoic acid (C15:0); (**7**) palmitic acid (C16:0); (**8**) palmitoleic acid (C16:1); (**9**) heptadecanoic acid (C17:0); (**10**) stearic acid (C18:0); (**11**) oleic acid (C18:1n9); (**12**) linoleic acid (C18:2n6c); (**13**) α-linolenic acid (C18:3n3); (**14**) arachidic acid (C20:0); (**15**) eicosanoic acid (C20:1c); (**16**) eicosadienoic acid (C20:2c); (**17**) eicosatrienoic acid + heneicosanoic acid (C20:3n3 + C21:0); (**18**) behenic acid (C22:0); (**19**) docosadienoic acid (C22:2); (**20**) tricosanoic acid (C23:0); (**21**) lignoceric acid (C24:0).

Table 3. Composition in Sugars of *R. micrantha* (Mean ± SD; $n = 9$)[a]

sugar (g/100 g dw)	petals	fertilized flowers	unripe hips	ripening hips	overripe hips
fructose	5.33 ± 0.17 b	3.51 ± 0.09 c	0.41 ± 0.00 d	3.33 ± 0.27 c	10.30 ± 0.02 a
glucose	4.36 ± 0.18 b	2.32 ± 0.07 d	0.35 ± 0.00 e	3.19 ± 0.29 c	9.98 ± 0.01 a
sucrose	2.27 ± 0.01 b	1.87 ± 0.04 c	1.39 ± 0.05 e	4.86 ± 0.03 a	1.77 ± 0.03 d
trehalose	1.10 ± 0.05 a	0.79 ± 0.01 b	0.39 ± 0.01 c	0.17 ± 0.01 d	0.16 ± 0.02 d
total sugars	13.06 ± 0.29 b	8.49 ± 0.06 d	2.53 ± 0.07 e	11.55 ± 0.53 c	22.21 ± 0.03 a

[a] dw: dry weight. In each row, different letters mean significant differences ($p < 0.05$).

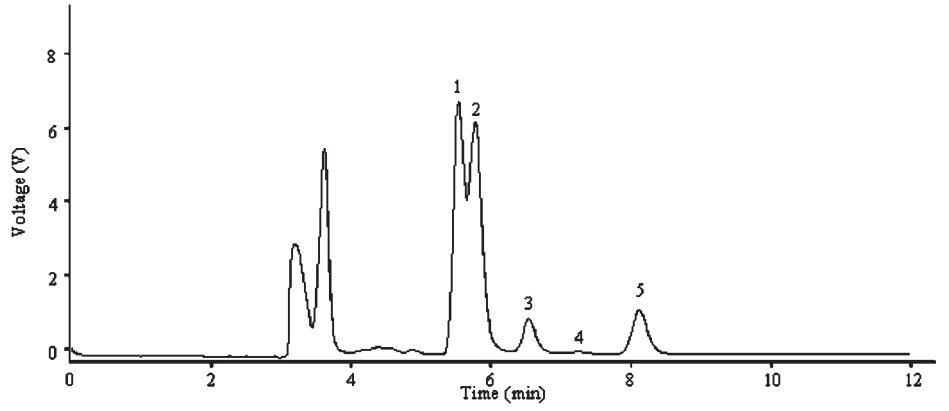

Figure 3. Individual sugars chromatogram of overripe hips of *R. micrantha*. (**1**) Fructose; (**2**) glucose; (**3**) sucrose; (**4**) trehalose; (**5**) IS (melezitose).

ascorbic acid in fruits of other rose species such as *R. canina*, *R. dumalis* subsp. *boissieri*, *R. dumalis* subsp. *antalyensis*, *R. villosa*, *R. pisiformis*, and *R. pulverulenta* (*4*) (726−943 mg/100 g). These values were similar to those found in the present study for ripe (943.89 mg/100 g) and overripe (693 mg/100 g) hips of *R. micrantha* (**Table 4**). Fruits from *R. rubiginosa* were described as having a high vitamin C content (400 mg/100 g), up to 10-fold higher than in orange juice (*9*) and 15 times higher than in citrus fruits (*15*). Moure et al. (*27*) reported that the vitamin C content of its fruits is 20−40 times higher than those in citrus fruits, although 95% of it is lost after drying for tea preparation.

Carotenoid pigments have been studied in many rose species, not only because of their participation in petal coloring but also for the intense color (from yellow to red) of the rose hips after blooming (*15*, *26*). Herein, β-carotene (46−62 mg/100 g dry weight) and lycopene (17−59 mg/100 g) were found in high levels, in overripe hips, ripening hips, and petals of *R. micrantha* (**Table 4**). Both compounds were quantified in similar amounts (49.76 and 39.19 mg/100 g for β-carotene and lycopene, respectively) in *R. rubiginosa* hips from Spain. These authors identified six major carotenoids (β-carotene, lycopene, rubixanthin, gazaniaxanthin, β-cryptoxanthin, and zeaxanthin) together with other minor carotenoids (violaxanthin, antheraxanthin, and γ-carotene) (*15*).

Table 4. Composition in Vitamins and Carotenoids of *R. micrantha* (Mean ± SD; $n = 9$)[a]

compd (mg/100 g dw)	petals	fertilized flowers	unripe hips	ripening hips	overripe hips
α-tocopherol	26.72 ± 0.44 b	33.45 ± 0.21 a	10.73 ± 1.12 d	18.17 ± 1.10 c	18.01 ± 0.40 c
β-tocopherol	0.72 ± 0.01 a	0.51 ± 0.02 b	nd	nd	0.12 ± 0.03 c
γ-tocopherol	7.68 ± 0.25 ba	7.31 ± 0.19 b	8.09 ± 0.01 a	1.47 ± 0.14 d	2.39 ± 0.01 c
δ-tocopherol	0.23 ± 0.02 b	0.81 ± 0.07 a	0.26 ± 0.01 b	nd	0.10 ± 0.01 c
total tocopherols	35.35 ± 0.68 b	42.08 ± 0.03 a	19.08 ± 1.10 c	19.64 ± 1.25 c	20.62 ± 0.45 c
ascorbic acid	295.08 ± 8.61 e	625.57 ± 10.75 c	580.49 ± 7.88 d	943.89 ± 7.39 a	693.69 ± 1.19 b
β-carotene	46.64 ± 0.05 c	2.26 ± 0.03 d	1.11 ± 0.02 e	57.66 ± 0.74 b	62.19 ± 0.09 a
lycopene	17.38 ± 0.05 c	1.63 ± 0.03 d	1.28 ± 0.05 d	18.35 ± 0.05 b	59.39 ± 0.60 a

[a] dw: dry weight; nd: not detected. In each row, different letters mean significant differences ($p < 0.05$).

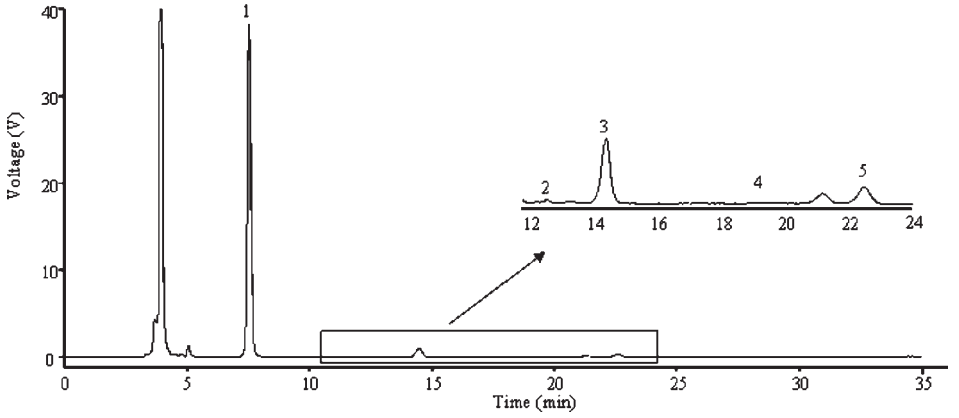

Figure 4. Individual tocopherols chromatogram of overripe hips of *R. micrantha*. (**1**) α-Tocopherol; (**2**) β-tocopherol; (**3**) γ-tocopherol; (**4**) δ-tocopherol; (**5**) IS (tocol).

Table 5. Extraction Yields, Composition in Phenolics and Flavonoids, and Antioxidant Activity EC_{50} Values of *R. micrantha* (Mean ± SD; $n = 9$)[a]

	petals	fertilized flowers	unripe hips	ripening hips	overripe hips
extraction yields (%)	36.64 ± 2.28	18.57 ± 0.99	25.16 ± 1.13	29.08 ± 1.20	35.62 ± 2.30
phenolics (mg GAE/g extract)	424.20 ± 31.77 b	527.07 ± 25.28 a	142.34 ± 3.45 e	188.16 ± 21.66 d	304.35 ± 34.16 c
flavonoids (mg CE/g extract)	78.46 ± 3.92 b	220.15 ± 1.89 a	12.69 ± 0.50 e	19.93 ± 0.81 d	65.09 ± 0.88 c
DPPH scavenging activity (μg/mL)	151.31 ± 10.71 cb	108.69 ± 1.05 c	1054.20 ± 112.62 a	204.23 ± 51.37 b	193.21 ± 43.27 b
reducing power (μg/mL)	53.79 ± 6.57 c	45.81 ± 12.08 c	674.33 ± 32.29 a	95.75 ± 6.81 b	79.06 ± 5.01 b
β-carotene bleaching inhibition (μg/mL)	39.26 ± 1.21 c	28.02 ± 0.82 d	174.29 ± 10.33 a	100.81 ± 6.14 b	44.25 ± 1.33 c
TBARS inhibition (μg/mL)	49.03 ± 5.84 c	35.84 ± 2.33 d	107.87 ± 12.89 a	72.89 ± 2.96 b	56.23 ± 3.99 c

[a] In each row, different letters mean significant differences ($p < 0.05$).

Herein, only β-carotene and lycopene were determined, but most probably other carotenoids such as the ones found in *R. rubiginosa* are also present in *R. micrantha*.

Antioxidant Activity. Phenolics and flavonoids varied in the same way for the different parts (**Table 5**); fertilized flowers revealed the highest content (527.07 and 220.15 mg/g extract, respectively), while unripe hips showed the lowest content (142.34 and 12.69 mg/g extract, respectively). Phenolics were also quantified in other rose species such as seeds of *R. rubiginosa* (*27*) and fruits of *R. canina*, *R. dumalis* subsp. *boissieri*, *R. dumalis* subsp. *antalyensis*, *R. villosa*, *R. pisiformis*, and *R. pulverulenta* (*4*), but the results were expressed in GAE per mass of material and not per mass of extract, as we decided to do for a better correlation to the antioxidant properties. No correlation between the extraction yield and the phenolics contents was observed.

All the samples proved to have antioxidant activity (**Table 5**) being more significant for fertilized flowers (lowest EC_{50} values). Unripe hips presented the lowest antioxidant properties (highest EC_{50} values), which are compatible to their lower phenolics and flavonoids content. All the parts, except unripe hips, showed better DPPH scavenging properties (50% at concentrations < 205 μg/mL) than methanolic and ethanolic extracts of *R. rubiginosa* seeds (52.2 and 80%, respectively, at 1000 μg/mL) (*27*). Other authors also stated high values of DPPH inhibition percentage of its seeds extracts that confirm *R. rubiginosa* as a good and cheap source of antioxidant substances (*9*).

DISCUSSION

Rosa micrantha is one of the rose species that grow wild in the northeastern Portuguese region so-called Nordeste Transmontano. Its traditional use is so claimed that this wild rose (as well as other wild species) became cultivated in almost every local home gardens and communitarian gardens, for easier use of its flowers, fruits, and galls and to enjoy it as ornamental and ritual plant (*21*). Beneficial health and cosmetic properties of *R. micrantha* flowers and hips have been transmitted by natives for centuries (*21*) but without a scientific support and an appropriate knowledge about the chemical constituents of all the used parts of this species.

Therefore, the chemical composition and bioactivity of different parts of *R. micrantha*, petals, fertilized flowers, unripe, ripening,

and overripe hips, were studied due to their medicinal and edible interest (*21*). These parts are mainly used to prepare homemade remedies that prevent or heal several human disorders and animal diseases (*20*, *21*). In Portugal, the use of *R. micrantha* hips as a source of nourishment is not so extended as other rose hips such as those from *R. canina* and *R. corymbifera*. However, several authors have documented the edible uses of flowers and hips from species of the section *Caninae* DC (*17*). Rose hips used to be eaten raw, ground and added to bread dough; petals and fruits are occasionally introduced in jam, jelly, and marmalade, brewed for tea, pressed and filtered to make rose hip syrup (*18*, *19*, *28*, *29*). Herein, the study of *R. micrantha* chemical composition proved the edible potential of ripening and overripe hips due to the presence of important metabolites such as proteins, carbohydrates, linoleic acid, ascorbic acid, and sugars, particularly the reducing sugars fructose and glucose.

The main fatty acids found in *R. micrantha*, α-linolenic and linoleic acids, are essential fatty acids as they cannot be synthesized by the human organism due to the lack of desaturase enzymes required for their production. They must be obtained by the diet and originate the omega-3 and omega-6 fatty acids, respectively. These omega fatty acids are the biosynthetic precursors of eicosanoids, meaning that their intake concentrations will strongly influence eicosanoids production, and, therefore, the organism's metabolic functions (*30*). Furthermore, these fatty acids, as also high ratios of PUFA/SFA and low n-6/n-3 ratios, can decrease the total amount of fat in blood (cholesterol), reducing the risk of cancer, cardiovascular, inflammatory, and autoimmune diseases (*31*, *32*). In fact, all the studied parts presented PUFA/SFA ratios higher than 0.45 and n-6/n-3 ratios lower than 4.0, which is recommended for the human diet (*33*) and makes *R. micrantha* a healthy food source. Moreover, the oil fraction (rich in oleic, linolenic, and linoleic fatty acids) of flowers or hips could find applications as an active part of cosmetic and pharmaceutical compositions like those found by other rose species such as *R. rubiginosa* (*10*−*13*).

Data in sugars composition are also in agreement with empirical knowledge and uses. According to some informants, children used to eat the fleshy part of the fully ripened hips pretending they were candies; mature hips were and still are processed in jams, syrups, and herbal teas (*21*, *28*, *29*).

Vitamin E is characteristic of fat-rich foods and not of fruits; nevertheless, the contents found in fertilized flowers and petals were not negligible. α- and γ-Tocopherols are the most abundant natural antioxidants and play a key role in DNA repair, immune function, and other physiological processes. Because of its role as a scavenger of free radicals, vitamin E is also believed to protect our bodies against degenerative malfunctions, mainly cancer and cardiovascular diseases (*34*). The high levels of tocopherols found in petals and fertilized flowers, and of ascorbic acid found in ripening and overripe hips, even higher than the content found in *R. rubiginosa* fruits that revealed 20−40 times more ascorbic acid than citrus fruits (*27*), make *R. micrantha* a promising source to be used in antiaging creams or in additives to preserve foods.

Overripe, ripening hips, and petals proved to be interesting sources of carotenoid pigments. In fact, rose hips have a characteristic intense reddish color due to carotenoids, and this fact makes *R. micrantha* a highly interesting and profitable source of these pigments for dying and pharmaceutical/additive food applications due to their pigmenting properties and antioxidant role in the cellular protection against lipid peroxidation, respectively.

The relationship between food and health becomes increasingly significant as consumers now demand healthy, tasty, and natural functional foods that have been grown in uncontaminated environments (*4*). Therefore, we decided to perform four different assays for the in vitro evaluation of the antioxidant properties of *R. micrantha*: scavenging activity on DPPH radicals (measuring the decrease in DPPH radical absorption after exposure to radical scavengers), reducing power (measuring the conversion of a Fe^{3+}/ferricyanide complex to the ferrous form), inhibition of β-carotene bleaching (by neutralizing the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models), and inhibition of lipid peroxidation in brain tissue (measured by the color intensity of MDA−TBA complex). These assays were performed using the whole extract, taking advantage of the complex mixture of phytochemicals with potential additive and synergistic effects (*35*), including phenolic compounds, particularly flavonoids. Different studies support the role of phenolics on human health by lowering the incidence of coronary heart disease and preventing thrombotic and atherogenic processes, as well as acting as antiviral agents against some diseases, such as diarrhea, arthritis, influenza, and poliomyelitis (*36*). Furthermore, their preservative properties on lipid-containing foods has found application in several commercial products like raw and roast ham, hamburgers, or fish (*9*, *37*).

R. micrantha showed antioxidant effects (particularly fertilized flowers and petals that revealed the highest antioxidant activity and the highest contents of tocopherols, phenolics, and flavonoids), which provide scientific evidence for their folk uses in the treatment of diseases related to the production of ROS and oxidative stress. Both petals and fertilized flowers were used to prepare rosewater by steeping in water and taking advantage of the natural fragrance of *R. micrantha*. Rosewater and the fertilized flower decoctions were usually provided for proper washing and skin care and were also available for the treatment of acne and for relieving irritated skin and eyes (*21*). Further experiments are warranted to explore its action mechanisms and reliable pharmaceutical and cosmetic applications.

The successful experience of propagating wild roses carried out by generations of informants can be useful in the implementation of sustainable cultivation of *R. micrantha* in the northeastern region of Portugal. It seems that wild roses are crops requiring low levels of maintenance (*38*) and therefore easily adapting to grow in most farmlands. As rose hips and products made from them have great potential because of their nutritional, health-promoting, and pharmaceutical properties, the plants growing feral can also be explored locally, as it is in the case of other wild species such as blackberries (*Rubus ulmifolius* Schott) and herbal teas (*Malva sylvestris* L. and *Hypericum perforatum*). In fact, the sustainable collection of wild species and fruits is already the base of several regional products and their certification as "Protected Designation of Origin" (PDO) and "Protected Geographical Indication" (PGI) contribute to local sustainable development.

Overall, the present work describes, for the first time, the chemical composition and bioactivity of *R. micrantha*. Powerful antioxidants and healthy compounds such as vitamins (ascorbic acid and tocopherols), carotenoids (β-carotene and lycopene), phenolics, flavonoids, reducing sugars, and essential fatty acids were quantified in this species. Furthermore, antioxidant activity data support *R. micrantha* folk applications and open possibilities of food, cosmetic, and pharmaceutical applications.

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