Nutritional composition and bioactivity of *Umbilicus rupestris* (Salisb.) Dandy: An underexploited edible wild plant

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ABSTRACT

The inclusion of edible wild plants in human diet has been receiving an increasing attention, as they represent an easily accessible source of nutrients, vitamins and antioxidants. In this work, the leaves of *Umbelicus rupestris* (Salisb.) Dandy, an edible species for which only scarce data is available in literature, were thoroughly evaluated for its nutritional profile, chemical characterization and bioactive properties. Being considered a succulent plant, the leaves revealed a very high content of moisture, with several beneficial compounds, including omega-3 polyunsaturated fatty acids, tocopherols and different polyphenols. A total of twelve flavonoids, three phenolic acids and one phenylpropanoid glucoside were identified in the decoction and/or hydroethanolic extracts, with most of them being described for the first time in this plant. Both extracts showed antioxidant activity and potential to inhibit some of the assayed bacteria, while not presenting cytotoxic effects on a non-tumour primary cell culture.

1. Introduction

Wild vegetables represent an important resource for humans worldwide, not only for their nutritional potential, as they are an inexpensive source of nutrients, vitamins, antioxidants and minerals, but also as a cultural heritage that should be protected (Ferreira, Morales, & Barros, 2016; Pardo-de-Santayana et al., 2007). In several areas of the globe, such as the Mediterranean region, different vegetables that grow spontaneously without being cultivated are still traditionally consumed and play an important role in the diet of local populations, in particular of those living in more rural areas. Nevertheless, this knowledge acquired during centuries and transmitted among generations, is facing an increasing risk of being lost due to a growing pressure of globalization, with some wild edible plants that were once used, now being rarely or no longer consumed (Geraci, Amato, Di Noto, Bazan, & Schicchi, 2018; Łuczaj et al., 2012; Pardo-de-Santayana et al., 2007). Fortunately, in the last years, wild edible plants have been receiving more attention not only from the scientific community, with the performance of ethnobotanical studies that allows documenting this ancient knowledge and studies comprising their chemical characterization, but also from the food industry and consumers, which are

increasingly interested in sustainable and healthy foods. Additionally, across Europe and several developed countries, a new trend has been recently emerging in nutrition and contemporary cuisine, leading to an increasing incorporation of local wild plants in modern dishes as an element of cultural identity but also seeking their health benefits (Geraci et al., 2018; Łuczaj et al., 2012).

Umbilicus rupestris (Salisb.) Dandy, commonly called navelwort or wall pennywort, is an edible wild plant that belongs to the Crassulaceae family, being characterized by a deformity in the leaf centre forming a navel (Gonzáles, Román, & Castro, 2009). *U. rupestris* is a succulent plant native from the Western Europe and the Mediterranean region, being frequently found in stone walls, cliffs and rock faces, preferring shaded and moist places (Afferni, 2009; Daniel, Woodward, Bryant, & Etherington, 1985). Due to the limited availability of soil in these habitats, the plant is often exposed to intermittent drought periods. When exposed to water stress, *U. rupestris* presents the capacity of switching from C3 photosynthesis to Crassulacean Acid Metabolism (CAM), a key adaptation typically found in a wide range of succulent plants, characterized by the closing of stomata during the day and opening at night when evaporative water loss is lower (Daniel et al., 1985).

Being a succulent plant, *U. rupestris* is mainly consumed raw in

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salads since its leaves have a fleshy crunchy texture (Geraci et al., 2018; Pardo-de-Santayana, Morales, Tardío, & Molina, 2018; Torija-Isasa & Matallana-González, 2016). Generally, the younger leaves are preferred for their mild flavour, especially in winter and early spring, because as the plant matures the leaves become bitter and not so pleasant-tasting. As happens with many plants from the Mediterranean flora, besides being used as food, ethnobotanical surveys have reported the usefulness of *U. rupestries* leaves in traditional medicine, namely against the inflammation and irritation of the skin, to treat furuncles, as disinfectant of wounds and with healing properties of burns (Benhouda & Yahia, 2015; Vickery, 2019). According to Allen and Hatfield (2004), in Britain and Ireland folk tradition, *U. rupestries* has been valued for soothing and healing soreness of the skin, calluses and as a treatment for various kinds of skin eruptions. The leaves can be applied as a poultice or reduced to a form in which it could be mixed with cream or fat and turned into an ointment. In some regions, it is also described its use as the pre-eminent salve for burns (Allen & Hatfield, 2004). The infusion prepared with the leaves has also been reported to have diuretic properties and can be used as ophthalmic disinfectant (Benhouda & Yahia, 2015; Gonzáles et al., 2009), while leaves prepared with olive oil were once used as an ointment to treat haemorrhoids (Carvalho & Morales, 2010). Despite its interest as both wild plant food and traditional medicine, as far as the authors know, studies focusing on the chemical characterization of this plant are almost inexistent, with no reports about its nutritional composition and only a few phytochemical compounds, namely three phenylpropanoids, being described so far (Viornery et al., 2000). Likewise, studies on the bioactive properties of this plant are very scarce on the literature (Benhouda & Yahia, 2015; Benhouda et al., 2014; Bullitta, Piluzza, & Manunta, 2013). Therefore, this work aims at the detailed chemical characterization of *U. rupestris* leaves regarding the nutritional value and phytochemical composition of samples collected in Portugal, in the same period at three different geographical locations. In addition, the bioactive potential of the hydroethanolic and decoctions extracts was also assessed in terms of its hepatotoxicity, antioxidant, anti-inflammatory and antimicrobial properties.

2. Materials and methods

2.1. Samples

Samples of *Umbilicus rupestris* (leaves) were harvested in January 2018 in different regions of Portugal, more specifically in the centre region (city of Viseu, 40° 39′ 39″ N, 7° 54′ 34″ E, 468 m a.s.l.), in the north inland region (near the city of Vila Real, 41° 17′ 34″ N, 7° 47′ 16″ W, 614 m a.s.l.) and in the northeast region (near the city of Bragança, 41° 54′ 04″ N, 6° 44′ 22″ W, 669 m a.s.l.). The samples from Viseu, Vila Real and Bragança were denominated as UR1, UR2 and UR3, respectively.

The fresh leaves were lyophilized (−49 °C, 0.08 bar, 48 h, FreeZone 4.5, Labconco, MO, USA) and reduced to a fine powder (20 mesh).

2.2. Hydroethanolic and decoction extracts

Hydroethanolic extracts were prepared by extracting 2.5 g of each freeze-dried sample with an ethanol:water solution (80:20, *v/v*) under magnetic stirring for 1 h. After filtration through a Whatman filter paper N°. 4, the plant residue was re-extracted and the combined filtrates were then evaporated under pressure at 40 °C (rotary evaporator Büchi R-210, Flawil, Switzerland) and subsequently lyophilized.

Decoctions were prepared using 1 g of freeze-dried samples and 100 mL of heated distilled water. The mixture was boiled for 5 min using a heating plate (VELP Scientific) and then filtrated through a Whatman filter paper N°. 4. The obtained decoctions were frozen and lyophilized to obtain a dried extract.

2.3. Chemical composition

2.3.1. Nutritional value

The proximate composition (moisture, fat, ash, proteins and carbohydrates) was determined in the freeze-dried samples, according to the AOAC methods (AOAC, 2016). The crude protein was determined by the macro-Kjeldahl method ($N \times 6.25$) using an automatic distillation and titration unit (model Pro-Nitro-A, JP Selecta, Barcelona), the crude fat was determined by Soxhlet extraction with petroleum ether during 7 h, and the ash content was evaluated by incineration at 550 ± 15 °C. Total carbohydrates content was calculated by difference using the formula: Total carbohydrates (g/100 g) = 100 − (g $_{fat}$ + g $_{ash}$ + g $_{proteins}$) and the energetic value was calculated according to the Atwater system using the formula: Energy (kcal/100 g) = 4 × (g $_{proteins}$ + g $_{carbohydrates}$) + 9 × (g $_{fat}$).

2.3.2. Free sugars

Soluble free sugars were determined on the freeze-dried plant (1.0 g) being extracted following a previously described methodology (Barros et al., 2013). The analyses were performed using a high performance liquid chromatography system coupled with a refraction index detector (HPLC-RI; Knauer, Smartline system 1000, Berlin, Germany) operating as previously described (Barros et al., 2013). Compounds were identified by comparison with standards and quantified by the internal standard (IS) method using melizitose. Results were recorded and processed using the Clarity 2.4 software (DataApex, Prague, Czech Republic) and expressed as g per 100 g of plant fresh weight.

2.3.3. Organic acids

Organic acids were extracted from the freeze-dried plant (1.0 g) using a previously described methodology (Barros et al., 2013) being subsequently analyzed using a ultra-fast liquid chromatography system coupled with a diode-array detector (UPLC-DAD; Shimadzu 20A series UFLC, Shimadzu Corporation, Kyoto, Japan) operating as previously described (Barros et al., 2013). Compounds were identified by comparison of retention time and spectra with those of standards (oxalic, quinic, malic, ascorbic, citric and fumaric acids, Sigma-Aldrich, St. Louis, MO, USA) and quantified based on calibration curves obtained by plotting the peak area recorded at 245 nm for ascorbic acid and at 215 nm for the remaining acids against concentration (mg/mL). The results were expressed as g per 100 g of plant fw.

2.3.4. Fatty acids

Fatty acids composition was determined by gas-liquid chromatography, after the extraction and derivatization to fatty acid methyl esters (FAME) according to a previously described methodology (Obodai et al., 2017). The analysis was carried out using a DANI model GC 1000 instrument equipped with a *split/splitless* injector set at 250 °C with a split ratio of 1:50, a flame ionization detector (FID) set at 260 °C and a Zebron-Kame column (30 m × 0.25 mm ID × 0.20 μm *df*, Phenomenex, Lisbon, Portugal). The following oven temperature program was used: initial temperature of 100 °C, held for 2 min, then a 10 °C/min ramp to 140 °C, 3 °C/min ramp to 190 °C, 30 °C/min ramp to 260 °C and held for 2 min. Hydrogen was used as the carrier gas with a flow-rate of 1.1 mL/min, measured at 100 °C. Fatty acid identification and quantification was performed by comparing the relative retention times of FAME peaks from samples with those of standards (reference standard mixture 47,885-U, Sigma, St. Louis, MO, USA). The results were recorded and processed using the Software Clarity DataApex 4.0 Software (Prague, Czech Republic) and expressed in relative percentage of each fatty acid.

2.3.5. Tocopherols

Tocopherols were determined on the freeze-dried plant (~500 mg) using a high performance liquid chromatography system coupled with a fluorescence detector (HPCL-FL; Knauer, Smartline system 1000, Berlin, Germany), as previously described (Barros, Carvalho, Morais, &

Ferreira, 2010). The quantification of the different tocopherol isoforms (α-, β-, γ- and δ-) was performed based on calibration curves constructed using authentic standards (Sigma, St. Louis, MO, USA) and tocol (Matreya, Pleasant Gap, PA, USA) as internal standard. The results were recorded and processed using the Clarity 2.4 software (DataApex, Prague, Czech Republic) and expressed in mg per 100 g plant fw.

2.3.6. Phenolic compounds

Phenolic compounds were determined in the hydroethanolic and decoction extracts, which were re-dissolved in methanol/water (80:20, *v/v*) to a final concentration of 5 mg/mL and filtered using a 0.22 μm disposable filter disk. The extracts were then evaluated using high performance liquid chromatography coupled with a diode-array and mass spectrometer detector (HPLC-DAD-ESI-MS/MS) operating under the conditions thoroughly described by the authors (Bessada, Barreira, Barros, Ferreira, & Oliveira, 2016). Compounds identification was performed by comparison of retention times, UV–VIS and spectra mass of samples compounds with those of the available standards. In the case standards were not available, compounds were tentatively identified based on the fragmentation pattern and data reported in the literature. Compounds were quantified as caffeic acid, ferulic acid and quercetin-3-*O*-glucoside by comparing the areas of the peaks recorded from samples with the calibration curves obtained from standards (200–5 μg/mL, Extrasynthese, Genay, France). Results were expressed in mg per g of extract.

2.4. Bioactive properties

2.4.1. Evaluation of in vitro antioxidant properties

The antioxidant activity of both the hydroethanolic and decoction extracts was determined in vitro according to the methodologies reported by the authors (Sarmento, Barros, Fernandes, Carvalho, & Ferreira, 2015) and included the following assays: DPPH (*2,2*-diphenyl-1-picrylhydrazyl, Alfa Aesar, Ward Hill, MA, USA) radical-scavenging activity, reducing power (measured by ferricyanide Prussian blue assay), β-carotene bleaching inhibition (assessed though the β-carotene/linoleate assay) and lipid peroxidation inhibition (assessed by the thiobarbituric acid reactive substances (TBARS) assay in porcine brain homogenates). The tested concentration of both hydroethanolic and decoction extracts ranged from 5 to 0.0195 mg/mL. EC_{50} values (sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the case of the reducing power assay) were used to expressed the results. Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard.

2.4.2. Hepatotoxic activity

Hepatotoxicity was evaluated using a primary cell culture prepared from porcine liver (PLP2), which was prepared according to the procedure described by the authors (Abreu et al., 2011). The tested concentration of both hydroethanolic and decoction extracts ranged from 400 to 6.5 μg/mL. The results were measured using the Sulforhodamine B (SRB) method and were expressed as GI_{50} values (concentration that inhibits 50% of cell growth).

2.4.3. Anti-inflammatory activity

The anti-inflammatory activity of the hydroethanolic and decoction extracts was determined based on the nitric oxide (NO) production by a murine macrophage (RAW 264.7) cell line, induced by the addition of lipopolysaccharide (LPS). The tested concentration of both hydroethanolic and decoction extracts ranged from 400 to 6.5 μg/mL. NO production was quantified based on nitrite concentration using the Griess Reagent System kit containing sulfanilamide, *N*-1-naphthylethylenediamine dihydrochloride and nitrite solutions following a procedure previously described by Correa et al. (2015). Dexamethasone was used as a positive control while no LPS was added in negative controls. The effect of the tested extracts in NO basal levels was also assessed by performing the assay in the absence of LPS. The results were expressed as IC_{50} values (μg/mL), corresponding to the extract concentration providing 50% inhibition of NO production.

2.4.4. Antimicrobial activity

The antimicrobial activity of the hydroethanolic and decoction extracts was determined against clinical isolates obtained from patients hospitalized in the Local Health Unit of Bragança and Hospital Center of Trás-os-Montes and Alto-Douro Vila Real, following the microdilution method described by the authors (Pires et al., 2018). The tested concentration of both hydroethanolic and decoction extracts ranged from 20 to 0.156 mg/mL. The tested microorganisms included Gram-positive (*Enterococcus faecalis*, *Listeria monocytogenes* and *methicillin-resistant Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Morganela morganii*, *Proteus mirabilis* and *Pseudomonas aeruginosa*). The measurement of the minimum inhibitory concentration (MIC) was determined by a colorimetric microbial viability assay based on the reduction of iodonitrotetrazolium chloride (0.2 mg/mL). The minimum bactericidal inhibitory concentration (MBC) was evaluated by plating a loopful of the content of the microwells that did not exhibited coloration in the MIC assay. Ampicillin and Imipenem were used as negative controls for Gram-negative bacteria while Vancomycin and Ampicillin were used for Gram-positive bacteria. Culture broth (Muller Hinton Broth added with 5% dimethylsulfoxide) inoculated with each bacteria was used as positive control.

2.5. Statistical analysis

Results were expressed as mean ± standard deviation (SD) of the analysis of three samples, each being assayed in triplicate. The statistical analysis was by using SPSS v. 23.0 software and comprised a one-variance analysis (ANOVA) followed by Tukey's HSD test (α = 0.05).

3. Results and discussion

3.1. Chemical composition

Considering that a range of different factors are known to affect the phytochemical profile of plants, including the geographical origin with its inherent variation of climatic conditions and soil characteristics, harvesting period, plant part and genetic factors, among others, in this study samples were collected from three distinct locations to account for geographical origin variability. The samples were all collected at the same harvesting period, with at least 300 g of young leaves being picked from each region (Viseu, UR1; Vila Real, UR2 and Bragança, UR3).

The results obtained for the proximal composition, free soluble sugars and organic acids is presented in Table 1. As expected due to the crassulacean acid metabolism (CAM) characteristic of succulent plants like *U. rupestris*, all samples presented a very high moisture content. As previously mentioned, CAM is characterized by the opening of stomas during the night and closure during most part of the day, allowing for a minimum loss of water (Geydan & Melgarejo, 2005). For the macronutrients, in all samples carbohydrates were found to be the major group, followed by proteins and ash, with lipids being the less abundant. Statistically significant differences ($p < 0.05$) were found for all groups, with exception for moisture. This can possibly be ascribed to environmental factors, such as climate and soil conditions. In particular, cold and hydric stress, can promote changes in the chemical composition of plants (Amaral et al., 2006; Machado et al., 2013). Observing the meteorological data in December for the three locations, Viseu presented the highest mean values of minimum temperature, maximum temperature and rainfall, followed by Vila Real, with Bragança presenting the lower minimum and rainfall values (de Clima, 2017) (Supplementary material, Fig. S1).

Table 1

Nutritional value, energetic value, free sugars and organic acids of the studied *Umbilicus rupestris* samples (mean ± SD, *n* = 3).

	UR1	UR2	UR3
Nutritional value			
Moisture	93 ± 1[a]	94.6 ± 0.5[a]	93.6 ± 0.1[a]
Ash (g/100 g fw)	0.91 ± 0.01[a]	0.61 ± 0.07[b]	0.83 ± 0.05[a]
Proteins (g/100 g fw)	1.83 ± 0.06[a]	1.60 ± 0.05[b]	1.35 ± 0.01[c]
Lipids (g/100 g fw)	0.255 ± 0.002[a]	0.203 ± 0.006[b]	0.17 ± 0.02[c]
Carbohydrates (g/100 g fw)	3.90 ± 0.03[b]	2.94 ± 0.09[c]	4.03 ± 0.05[a]
Energy (kcal/100 g fw)	25.2 ± 0.1[a]	20.0 ± 0.2[c]	23.0 ± 0.1[b]
Free sugars (g/100 g fw)			
Manose	0.902 ± 0.01[a]	0.51 ± 0.02[c]	0.78 ± 0.06[b]
Sucrose	0.082 ± 0.002[a]	0.020 ± 0.002[c]	0.073 ± 0.003[b]
Total free sugars	0.98 ± 0.01[a]	0.53 ± 0.03[c]	0.85 ± 0.06[b]
Organic acids (g/100 g fw)			
Oxalic acid	0.48 ± 0.01[a]	0.33 ± 0.01[b]	0.27 ± 0.01[c]
Malic acid	0.210 ± 0.005[b]	0.215 ± 0.001[b]	0.421 ± 0.002[a]
Ascorbic acid	0.021 ± 0.001[a]	0.008 ± 0.001[c]	0.013 ± 0.001[b]
Citric acid	0.334 ± 0.001[b]	0.375 ± 0.002[a]	0.21 ± 0.01[c]
Fumaric acid	tr	tr	tr
Total organic acids	1.04 ± 0.01[a]	0.93 ± 0.01[b]	0.92 ± 0.01[b]

tr – traces; fw – fresh weigh. Different letters in the same row mean significant differences (*p* < 0.05). Samples were collected from different regions, UR1:Viseu, UR2: Vila Real, UR3: Bragança.

Only two soluble sugars were identified in the three samples, namely manose and sucrose, with total free sugars ranging from 0.53 to 0.98 g per 100 g of fresh plant (Table 1). Moreover, the leaves of *U. rupestris* showed a low energetic value, therefore being adequate to be included in low carb diets.

Regarding organic acids, four compounds were identified, namely oxalic, malic, ascorbic and citric acids, although trace levels of fumaric acid were also detected in the three analysed *U. rupestris* leaves (Table 1). As referred, although *U. rupestris* can behave as a normal C-3 plant, it can change to CAM in water stress conditions, which is usual to occur in this plant since it mainly grows on walls, cliffs and rock-faces with very few soil to retain water (Daniel et al., 1985). A feature of CAM plants is that during the night they produce oxaloacetate which is rapidly converted to malate, thus explaining that these two acids were detected as major organic acids in samples UR1 and UR3. Interestingly, samples UR1 and UR2 presented higher levels of malic acid compared to citric acid, while the opposite was observed in UR3. According to Chen and Nose (2004) the transport of malate to the vacuoles is associate to CAM metabolism and requires the use of energy, therefore implying the consumption of citric acid and explaining the lower level of this acid in the sample UR3, which was the one with higher malic acid content. During the day, malate is decarboxylated and the CO_2 is re-fixed via the Calvin cycle, therefore causing a diurnal fluctuation in acid content of the leaves (Daniel et al., 1985). For this reason, the collection hour of the samples was carefully chosen, with all being harvested in the afternoon (between 16:00 and 17:00H). Even so, statistically significant differences were found to occur among the three samples, which can be due to other environmental or even genetic factors.

Determination of plants fatty acids composition enables a more detailed evaluation of the nutrients composition and potential benefits related to their consumption. The fatty acid profile of *U. rupestris* leaves is shown in Table 2, evidencing that a total of 24 fatty acids were identified, with the two major compounds being the essential fatty acids α-linolenic and linoleic acids. In the three evaluated samples, polyunsaturated fatty acids (PUFA) were the major group, with large predominance of ω-3 over ω-6 fatty acids. An adequate intake of ω-3 fatty acids is of great importance as they can influence inflammation, blood pressure, vasoconstriction, blood clotting and immune function,

Table 2

Composition of fatty acids and of tocopherols of the studied *Umbilicus rupestris* samples (mean ± SD, *n* = 3).

	UR1	UR2	UR3
Fatty acids			
C8:0	0.043 ± 0.001[a]	0.025 ± 0.001[c]	0.029 ± 0.001[b]
C10:0	0.024 ± 0.001[a]	0.023 ± 0.002[a]	0.017 ± 0.001[b]
C11:0	0.23 ± 0.01[a]	0.18 ± 0.01[b]	0.26 ± 0.03[a]
C12:0	0.043 ± 0.001[c]	0.070 ± 0.001[a]	0.056 ± 0.002[b]
C13:0	0.169 ± 0.004[b]	0.38 ± 0.03[a]	0.12 ± 0.01[c]
C14:0	0.48 ± 0.007[b]	0.77 ± 0.02[a]	0.76 ± 0.05[a]
C14:1	0.153 ± 0.001[b]	0.275 ± 0.004[a]	0.086 ± 0.003[c]
C15:0	0.068 ± 0.005[b]	0.079 ± 0.003[a]	0.065 ± 0.001[b]
C16:0	10.6 ± 0.8[b]	12.0 ± 0.5[a]	11.0 ± 0.2[ab]
C16:1	1.07 ± 0.03[a]	0.98 ± 0.04[b]	0.61 ± 0.04[c]
C17:0	0.128 ± 0.001[b]	0.114 ± 0.008[c]	0.201 ± 0.001[a]
C18:0	1.6 ± 0.2[a]	0.80 ± 0.02[c]	1.06 ± 0.02[b]
C18:1n9	0.641 ± 0.002[b]	0.50 ± 0.02[c]	0.69 ± 0.02[a]
C18:2n6	18.3 ± 0.6[b]	17.9 ± 0.9[b]	21.0 ± 0.3[a]
C18:3n3	62 ± 2[a]	61 ± 2[ab]	58.9 ± 0.6[b]
C20:0	0.68 ± 0.04[a]	0.54 ± 0.04[b]	0.48 ± 0.02[b]
C20:1	0.037 ± 0.001[b]	0.047 ± 0.001[a]	0.028 ± 0.001[c]
C20:2	0.079 ± 0.004[b]	0.105 ± 0.005[a]	0.077 ± 0.003[b]
C21:0	0.046 ± 0.004[a]	0.035 ± 0.003[b]	0.028 ± 0.002[c]
C20:4n6	0.449 ± 0.004[c]	0.60 ± 0.02[a]	0.56 ± 0.02[b]
C20:3n3	0.151 ± 0.009[a]	0.11 ± 0.01[b]	0.121 ± 0.001[b]
C22:0	1.27 ± 0.09[a]	1.2 ± 0.1[a]	0.92 ± 0.05[b]
C23:0	1.25 ± 0.09[c]	1.83 ± 0.03[b]	2.038 ± 0.001[a]
C24:0	0.926 ± 0.002[ab]	0.9 ± 0.1[b]	0.95 ± 0.02[a]
SFA (%)	18 ± 1[a]	18.9 ± 0.7[a]	18.0 ± 0.3[a]
MUFA (%)	1.90 ± 0.02[a]	1.79 ± 0.05[b]	1.41 ± 0.01[c]
PUFA (%)	80 ± 1[a]	79.2 ± 0.8[a]	80.6 ± 0.3[a]
Tocopherols (μg/100 g fw)			
α-Tocopherol	636 ± 0.006[b]	552 ± 0.001[c]	700 ± 0.001[a]
β-Tocopherol	42 ± 0.001[a]	20 ± 0.001[c]	25 ± 0.001[b]
γ-Tocopherol	70 ± 0.001[c]	300 ± 0.001[a]	152 ± 0.001[b]
δ-Tocopherol	36 ± 0.002[c]	180 ± 0.001[a]	101 ± 0.003[b]
Total tocopherols (μg/100 g fw)	790 ± 0.01[c]	1050 ± 0.01[a]	980 ± 0.01[b]

Caprylic acid (C8:0); Capric acid (C10:0); Undecanoic acid (C11:0); Lauric acid (C12:0); Tridecanoic acid (C13:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1c); *cis*-11,14-Eicosadienoic acid (C20:2c); Heneicosanoic acid (C21:0); Arachidonic acid (C20:4n6); *cis*-11,14,17-Eicosatrienoic acid (C20:3n3); Behenic acid (C22:0); Tricosanoic acid (C23:0); Lignoceric acid (C24:0); SFA: Saturated fatty aids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. Different letters in the same row mean significant differences (*p* < 0.05).

among other processes (Calder, 2015). Also, dietary consumption of ω-3 PUFA is generally recommended in international guidelines for the general population to prevent the occurrence of cardiovascular diseases (CVD) since several large-scale randomized clinical trials have been showing that dietary intake of these compounds improves the prognosis of patients with CVD (Endo & Arita, 2016). The qualitative profile of the three samples was found to be identical and, although the content of some individual fatty acids was statistically different, no differences were observed among samples regarding the total PUFA and total saturated fatty acids (SFA) contents (*p* > 0.05).

Vitamin E is a term used to designate a family of related compounds, namely α-, β-, γ- and δ-tocopherols and tocotrienols, which present a common structure with a chromanol ring and an isoprenic side chain and whose biological activities are mainly related to their antioxidant activity, enabling them to inhibit lipid peroxidation in biological membranes (Tucker & Townsend, 2005; Woollard & Indyk, 2003). Vitamin E composition of the studied samples is shown in Table 2 were it can be observed that α-tocopherol was the major compound, followed by γ- and δ-tocopherols. All four isoforms of tocopherol were present in
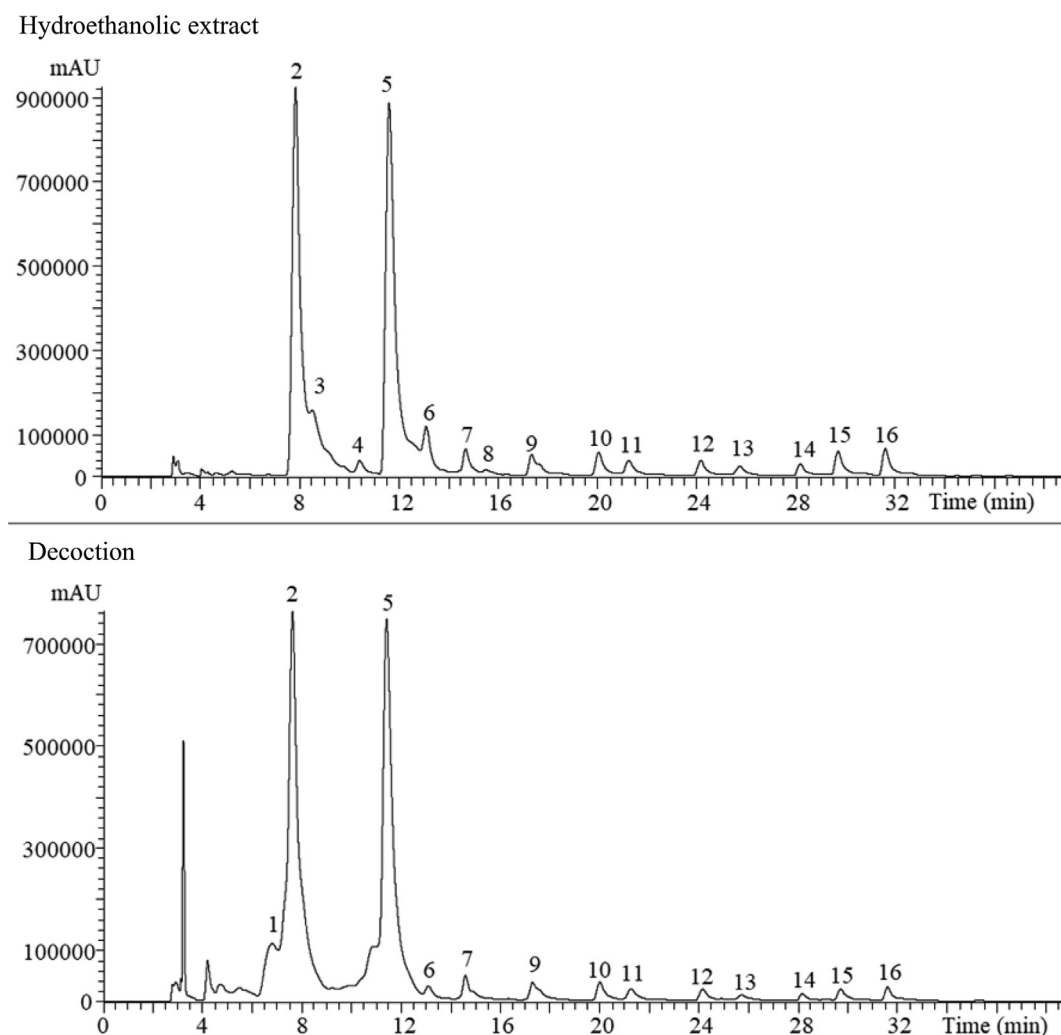
Hydroethanolic extract

Decoction

Fig. 1. Phenolic profile of the hydroethanolic and decoction extracts of *Umbilicus rupestris* recorded at 370 nm. The numbers shown in the chromatograms correspond to the compounds identified in Table 3.

the three samples from different geographical origins, although statistically significant differences were observed among them.

The characterization of *U. rupestris* phenolic compounds profile was performed by HPLC-DAD-ESI/MS (Fig. 1). Chromatographic data regarding the retention time, λ_{max}, pseudomolecular ion, main fragment ions in MS^2, tentative identification and quantification of the phenolic compounds are presented in Table 3. To the best of the authors knowledge, so far only one study has been performed regarding the phenolic profile of this species (Table 3), reporting the presence of three compounds, 2-O-caffeoyl malate, isoquercitrin and venusol (Viornery et al., 2000). In this study, *U. rupestris* sample revealed the presence of sixteen compounds, allowing us to confirm the presence of venusol in this species, and additionally describing the presence of three phenolic acids (hydroxycinnamic derivatives) and twelve flavonoids (flavonol and flavone glycoside derivatives). Compounds 3 (caffeic acid) and 8 (ferulic acid) were positively identified in comparison with the commercial standards. Compound 4 ([M−H]$^-$ at *m/z* 355) revealed 162 u (glycosil moiety) higher than peak 8, being identified as ferulic acid hexoside. Compound 6 ([M−H]$^-$ at *m/z* 323) was identified as venusol, taking into account its UV spectra and pseudomolecular ion pattern previously described for this compound by Viornery et al. (2000).

The remaining compounds were identified as flavonol glycoside derivatives: quercetin (λ_{max} around 353 nm, and an MS^2 fragment at *m/z* 301), isorhamnetin (λ_{max} around 358 nm, and an MS^2 fragment at

m/z 315), myricetin (λ_{max} around 356 nm, and an MS^2 fragment at *m/z* 317) and flavone glycoside derivatives: chrysoeriol (λ_{max} around 354 nm, and an MS^2 fragment at *m/z* 299). Compounds 1 and 2 presented the same pseudomolecular ion ([M−H]$^-$ at *m/z* 655), with MS^2 fragments revealing the alternative loss of glucuronyl (*m/z* at 479; −176 u) and hexosyl (*m/z* at 317; −162 u) residues, indicating location of each residue on different positions of the aglycone, being both identified as myricetin-O-glucuroside-O-hexoside. Similarly, compound 5 ([M−H]$^-$ at *m/z* 639) was identified as quercetin-O-glucuroside-O-hexoside. Compound 7 ([M−H]$^-$ at *m/z* 741) presented 86 u (malonyl moiety) higher then compounds 1 and 2, being tentatively assigned as myricetin-O-malonylglucuroside-O-hexoside. The mass characteristics of peak 9 ([M−H]$^-$ at *m/z* 725) and 11 ([M−H]$^-$ at *m/z* 739) indicated that it corresponds to isorhamnetin derivatives, bearing in the first compound a pentosyl moiety (peak 9) and a deoxyhexoside in the second compound (peak 11), and a further loss in both peaks of a deoxyhexosyl-pentoside residues. The observation of MS^2 fragments at *m/z* 593 (−132 and 146 u) and 315 (−146 + 132 u), also indicated the alternative loss of each of the group of sugar moieties, respectively, pointing to their location on different positions of the aglycone. Thus, these compounds were tentatively identified as isorhamnetin-O-pentoside-O-(deoxyhexosyl-pentoside) and isorhamnetin-O-deoxyhexoside-O-(deoxyhexosyl-pentoside), respectively. Similarly, compounds 10 ([M−H]$^-$ at *m/z* 709) and 12 ([M−H]$^-$ at *m/z* 723) were tentatively identified as chrysoeriol-O-pentoside-O-(deoxyhexosyl-pentoside) and

Table 3

Identification and quantification of phenolic compounds (mg/g extract) in the hydroethanolic and decoction extracts of *U. rupestris*.

Peak	Rt (min)	λmax (nm)	[M−H]− (m/z)	MS² (m/z)	Tentative identification	UR1		UR2		UR3	
						Hydroethanolic	Decoction	Hydroethanolic	Decoction	Hydroethanolic	Decoction
1	6.8	355	655	479(100), 317(60)	Myricetin-O-glucuroside-O-hexoside	nd	0.62 ± 0.01[b]	nd	0.426 ± 0.001[c]	nd	0.79 ± 0.01[a]
2	7.6	358	655	479(100), 317(71)	Myricetin-O-glucuroside-O-hexoside	2.586 ± 0.005[b]	2.06 ± 0.02[c]	0.92 ± 0.04[e]	1.07 ± 0.04[d]	2.67 ± 0.01[a]	1.99 ± 0.02[c]
3	8.5	330	179	135(100)	Caffeic acid*	1.43 ± 0.04[b]	nd	2.3 ± 0.2[a]	nd	1.211 ± 0.06[c]	nd
4	10.4	318	355	193(67),133(100)	Ferulic acid hexoside**	0.080 ± 0.002[a]	nd	0.091 ± 0.001[a]	nd	0.059 ± 0.019[b]	nd
5	11.4	356	639	463(100), 301(59)	Quercetin-O-glucuroside-O-hexoside	2.83 ± 0.03[b]	2.40 ± 0.02[c]	1.69 ± 0.09[e]	1.804 ± 0.004[d]	3.337 ± 0.004[a]	2.42 ± 0.04[c]
6	13.1	326	323	179(29),175(21),163(20),159(6), 147(13),133(62),123(25),105(100)	Venusol	nq	nq	nq	nq	nq	nq
7	14.6	354	741	479(100),317(42)	Myricetin-O-malonylglucuroside-O-hexoside	0.310 ± 0.001[cd]	0.318 ± 0.002[c]	0.30 ± 0.01[d]	0.283 ± 0.003[f]	0.366 ± 0.001[a]	0.320 ± 0.002[c]
8	15.5	324	193	134(100)	Ferulic acid**	0.082 ± 0.001[a]	nd	0.073 ± 0.002[b]	nd	0.072 ± 0.002[b]	nd
9	17.3	360	725	593(100),315(50)	Isorhamnetin-O-pentoside-O-(deoxyhexosyl-pentoside)	0.295 ± 0.001[a]	0.260 ± 0.001[b]	0.208 ± 0.001[d]	0.201 ± 0.002[e]	0.261 ± 0.001[b]	0.248 ± 0.001[c]
10	20.0	354	709	577(100),299(69)	Chrysoeriol-O-pentoside-O-(deoxyhexosyl-pentoside)	0.328 ± 0.001[a]	0.269 ± 0.001[e]	0.277 ± 0.006[d]	0.258 ± 0.006[f]	0.347 ± 0.001[a]	0.310 ± 0.001[c]
11	21.3	360	739	593(100),315(54)	Isorhamnetin-O-deoxyhexoside-O-(deoxyhexosyl-pentoside)	0.2820 ± 0.0005[a]	0.249 ± 0.002[c]	0.221 ± 0.002[d]	0.207 ± 0.001[e]	0.265 ± 0.006[b]	0.2529 ± 0.0005[c]
12	24.1	354	723	577(100),299(77)	Chrysoeriol-O-deoxyhexoside-O-(deoxyhexosyl-pentoside)	0.2788 ± 0.0002[a]	0.243 ± 0.001[d]	0.218 ± 0.002[e]	0.211 ± 0.002[f]	0.273 ± 0.001[b]	0.253 ± 0.001[c]
13	25.7	360	811	593(100),315(51)	Isorhamnetin-O-malonylpentoside-O-(deoxyhexosyl-pentoside)	0.252 ± 0.004[a]	0.218 ± 0.001[c]	0.212 ± 0.001[d]	0.204 ± 0.001[e]	0.2252 ± 0.0005[b]	0.212 ± 0.002[d]
14	28.2	354	795	577(100),299(71)	Chrysoeriol-O-malonylpentoside-O-(deoxyhexosyl-pentoside)	0.256 ± 0.002[b]	0.2186 ± 0.0004[e]	0.248 ± 0.003[c]	0.2194 ± 0.0001[e]	0.272 ± 0.001[a]	0.242 ± 0.002[d]
15	29.7	360	825	593(100),315(43)	Isorhamnetin-O-malonyldeoxyhexoside-O-(deoxyhexosyl-pentoside)	0.330 ± 0.003[a]	0.2402 ± 0.0002[c]	0.215 ± 0.002[e]	0.201 ± 0.002[f]	0.261 ± 0.003[b]	0.230 ± 0.001[d]
16	31.6	354	809	577(100),299(56)	Chrysoeriol-O-malonyldeoxyhexoside-O-(deoxyhexosyl-pentoside)	0.342 ± 0.003[a]	0.241 ± 0.003[d]	0.246 ± 0.002[d]	0.217 ± 0.002[e]	0.331 ± 0.005[b]	0.274 ± 0.003[c]
Total phenolic acids						1.59 ± 0.04[b]	nd	2.5 ± 0.2[a]	nd	1.34 ± 0.04[c]	nd
Total flavonoids						8.08 ± 0.02[b]	7.34 ± 0.01[d]	4.8 ± 0.1[f]	5.30 ± 0.04[e]	8.61 ± 0.01[a]	7.55 ± 0.01[c]
Total phenolic compounds						9.68 ± 0.06[a]	7.34 ± 0.01[b]	7.2 ± 0.3[d]	5.30 ± 0.04[c]	9.95 ± 0.04[a]	7.55 ± 0.01[b]

nq – not quantified; nd - not detected. Calibration curves standards: *caffeic acid (y = 388345x + 406,369, R² = 0.994); **ferulic acid (y = 633,126x − 185,462; R² = 0.999); all the other compounds were quantified as quercetin-3-O-glucoside (y = 34843x − 160,173, R² = 0.998). UR1, UR2 and UR3: samples from different geographic origins. Different letters in the same row mean significant differences (p < 0.05).

chrysoeriol-*O*-deoxyhexoside-*O*-(deoxyhexosyl-pentoside), respectively.

Compounds 13 ([M−H]⁻ at *m/z* 811) and 15 ([M−H]⁻ at *m/z* 825) presented 86 u (malonyl moiety) higher then compounds 9 and 11, being tentatively assigned as isorhamnetin-*O*-malonylpentoside-*O*-(deoxyhexosyl-pentoside) and isorhamnetin-*O*-malonyldeoxyhexoside-*O*-(deoxyhexosyl-pentoside), respectively. Taking into account these findings, compounds 14 ([M−H]⁻ at *m/z* 795) and 16 ([M−H]⁻ at *m/z* 809) were also identified as having malonyl residues (86 u) of compounds 10 and 12, thus being tentatively assigned as chrysoeriol-*O*-malonylpentoside-*O*-(deoxyhexosyl-pentoside) and chrysoeriol-*O*-malonyldeoxyhexoside-*O*-(deoxyhexosyl-pentoside), respectively.

In general, hydroethanolic extracts presented higher amounts of phenolic compounds in comparison with decoctions. These last extracts presented only flavonoid compounds with phenolic acids being absent, possibly because of degradation during the heating process. Myricetin-*O*-glucuroside-*O*-hexoside (compound 2) and quercetin-*O*-glucuronide-*O*-hexoside (compound 5) were the most abundant compounds found in all samples and also considering both extracts. Comparing the three samples collected in the same period, but in different geographical locations, UR2 samples presented a statistically significant lower value of total phenolic compounds, when compared to UR1 and UR3 samples, which presented both a similar value. As mentioned, Viseu (UR1) and Bragança (UR3) regions presented, respectively, the highest and lowest mean values of minimum temperature, maximum temperature and rainfall, therefore suggesting that these parameters were not the most relevant in what concerns phenolic compounds composition. The quantitative difference observed for the Vila Real (UR2) sample may thus be explained by other factors such as soil composition and exposure to sunlight, or even due to genetic differences.

3.2. Bioactive properties of U. rupestris extracts

The results of the antioxidant activity assays obtained for the two extracts (hydroethanolic and decoction) prepared with the plants harvested from different locations, are shown in Table 4. As it can be observed, significant differences were found among the samples as well as between extraction methods. Independently of the assay considered, and for the three samples, better results were obtained for the hydroethanolic extracts as consistently this extraction method gave the lowest values of EC_{50}. This can possibly be ascribed to the presence of phenolic acids in all the hydroethanolic extracts comparatively to the respective decoction extracts where these compounds were not detected (Fig. 1 and Table 3). Moreover, for UR1 and UR3 samples, the content of flavonoids was higher in the hydroethanolic extract in comparison to the decoction, thus suggesting an influence of *U. rupestris* flavonoids on

the antioxidant activity properties of these extracts. Likewise, when comparing the results obtained for the hydroethanolic extracts of the three samples, UR2 was the one that presented the best results for the DPPH scavenging assay (1.39 mg/mL compared to 2.08 mg/mL and 2.7 mg/mL for UR3 and UR1, respectively), which can possibly be related to its much higher content in caffeic acid (2.3 mg/g extract) compared to UR1 and UR2 (1.4 and 1.2 mg/g extract, respectively). In previous studies, caffeic acid has been shown to be an effective antioxidant in different antioxidant assays, showing higher DPPH radical scavenging activity then butylated hydroxyanisole (BHA), α-tocopherol and trolox and higher reducing power compared to butylated hydroxytoluene, BHA, α-tocopherol, and Trolox (Gülçin, 2006). Overall, UR1 and UR2 hydroethanolic were the samples that revealed the highest antioxidant activity, which could be related to their higher content in phenolic compounds.

Until the present date, only a few studies have been conducted on the antioxidant properties of *U. rupestris*. Piluzza and Bullitta (2011) evaluated the antioxidant activity by means of ABTS⁺ and DPPH radical scavenging assays of twenty-four plant species of traditional ethnoveterinary medicine in the Mediterranean region and found *U. rupestris* as being among the seven plants that showed the highest antioxidant activity. The authors evaluated an extract obtained from the whole plant extracted with a mixture of acetone/water (7:3) and reported a value for the DPPH assay of 36.22 ± 2.61 mmol Trolox equivalents/100 g dry weight. Bullitta et al. (2013) evaluated the cellular antioxidant capability of eleven Mediterranean plant species related to ethnobotanic traditions, including *U. rupestris*, by assessing the capacity of inhibiting the formation of intracellular reactive oxygen species (ROS) in primary human umbilical vein endotelial cord blood cells (HUVEC) and in undifferentiated human promyelocytic leukemia cell-line (HL-60) as well as determining their antioxidant activity by chemical in-vitro assays (DPPH and ABTS). The extract of the whole *U. rupestris* plant prepared in dimethylsulfoxide (DMSO) showed a moderate attenuation of ROS generation in cell-culture, although being less effective in the endothelial cells compared to the promyelocytic leukemic cell-line, and presented an EC_{50} value of 47.4 ± 1.22 µg/mL in the DPPH radical scavenging assay (Bullitta et al., 2013).

Considering the described usage of *U. rupestris* leaves in traditional medicine, the prepared extracts were also evaluated for their anti-inflammatory properties, besides their cytotoxic potential in non-tumour cells. As it can be observed in Table 4, none of the extracts presented toxicity towards the liver primary cell culture (PLP2). Similar results have been reported in previous works, since the DMSO extract of *U. rupestris* whole plant (50 µg/mL) also did not show any putative toxic effect in HL-60 and HUVEC cells (Bullitta et al., 2013). Also, in acute toxicity studies carried out *in vivo* on Wistar rats, the median lethal dose

Table 4
Antioxidant activity, hepatotoxicity and anti-inflammatory activity of the hydroethanolic and decoction extracts of *U. rupestris* (mean ± SD, *n* = 3).

	UR1		UR2		UR3	
	Hydroethanolic	Decoction	Hydroethanolic	Decoction	Hydroethanolic	Decoction
Antioxidant activity (EC_{50} mg/mL)						
DPPH scavenging activity[*]	2.70 ± 0.09[c]	2.98 ± 0.06[a]	1.39 ± 0.09[f]	2.22 ± 0.03[d]	2.08 ± 0.07[e]	2.80 ± 0.06[b]
Ferricyanide/Prussian blue[**]	0.422 ± 0.002[f]	1.26 ± 0.08[d]	0.746 ± 0.008[e]	2.49 ± 0.06[b]	1.57 ± 0.02[c]	2.57 ± 0.05[a]
β-carotene/linoleate[*]	0.55 ± 0.03[d]	2.1 ± 0.2[c]	0.46 ± 0.03[d]	3.6 ± 0.2[a]	0.55 ± 0.02[d]	3.3 ± 0.3[b]
TBARS[*]	0.155 ± 0.005[e]	0.22 ± 0.03[c]	0.18 ± 0.01[d]	0.41 ± 0.02[a]	0.12 ± 0.01[f]	0.35 ± 0.02[b]
Hepatotoxicity (GI_{50} µg/mL)						
PLP2	> 400	> 400	> 400	> 400	> 400	> 400
Anti-inflammatory activity (EC_{50} µg/mL)						
NO-production[***]	> 400	> 400	> 400	> 400	> 400	> 400

EC_{50}: extract concentration corresponding to [*]50% of antioxidant activity or [**]0.5 of absorbance in the reducing power assay. Trolox EC_{50} values: 42 µg/mL (DPPH scavenging activity), 41 µg/mL (reducing power), 18 µg/mL (β-carotene bleaching inhibition) and 23 µg/mL (TBARS inhibition). GI_{50}: extract concentration responsible for 50% inhibition of growth in a primary culture of liver cells (PLP2). GI_{50} values for Ellipticine (positive control): 3 µg/mL. [***]EC_{50} values correspond to the extract concentration achieving 50% of the inhibition of NO-production. EC_{50} values for dexamethasone (positive control): 16 µg/mL. UR1, UR2 and UR3: samples from different geographic origins. Different letters in the same row mean significant differences (*p* < 0.05).

(LD$_{50}$) of the methanolic extract of *U. rupestris* was found to be higher than the maximum concentration tested (2 g/kg body weight), with this dose causing neither death or signs of toxic manifestations on the treated rats (Benhouda & Yahia, 2015). Regarding the anti-inflammatory properties, for the range of tested concentrations (up to 400 μg/mL) the results indicated the absence of activity in LPS-activated murine macrophages since no decrease of nitric oxide levels was observed (Table 4). Nevertheless, in previous works Benhouda and Yahia (2015) reported that the methanolic extract of *U. rupestris* leaves (100 and 200 mg/kg rat body weight) significantly inhibited, in a dose dependent manner, the inflammation induced in Wistar rats paws by the subcutaneous administration of carrageenan, chemical mediators (histamine and serotonin) and formalin. The authors suggested that the anti-inflammatory activity of the extract could be due to the inhibition of inflammation mediators, inhibition of cyclooxygenase and prostaglandin synthesis or inhibition of leukocytes migration. Therefore, the absence of activity in the present study can be either due to the low range of concentrations tested or to the selected assay, since the mechanism of action is different from the tested one, which can lay behind the plant's anti-inflammatory activity. In addition, other compounds present in the leaves of *U. rupestris,* namely the polysaccharide fraction, could also possibly support the empiric use of the plant for its anti-inflammatory properties. Although scarce studies have been carried out with plants from the Crassulaceae family, Sendl, Mulinacci, Vincieri, and Wagner (1993) have showed that two major rhamnogalacturonan polysaccharides isolated from the leaves of *Sedum telephium*, a succulent plant from the mentioned family also used in traditional medicine to treat skin inflammation, eczemas, wounds and burns, showed a high anti-inflammatory and immunological activity.

Table 5 shows the results of the antimicrobial activity of the hydroethanolic and decoction extracts of *U. rupestris* leaves against three Gram-positive and five Gram-negative multi-resistant pathogenic strains, isolated from hospitalized patients. The results showed that, in the tested concentrations (20–0.156 mg/mL), both extracts were ineffective against *P. mirabilis* and *P. aeruginosa*, however being active or moderately active against the remaining bacteria. Although some variability was found regarding extracts and samples from different geographical origins, in general, decoction extracts were more effective compared to hydroethanolic extracts, with the exception of UR3 hydroethanolic extract against *K. pneumoniae*, UR2 hydroethanolic extract against MRSA and *L. monocytogenes*. The lowest MIC values (5 mg/mL) were obtained against the Gram-negative *E. coli* and the Gram-positive *L. monocytogenes*. Curiously, in general, comparing the results obtained for the samples collected in three distinct geographical locations, better antimicrobial activity was found for UR2 decoction (lower MIC value for *K. pneumoniae*, *M. morganii*, *E. faecalis* and *L. monocytogenes*), even though this being the sample and extract method with the lowest value of total phenolic compounds (Table 3).

The antimicrobial activity of *U. rupestris* leaves methanolic extracts against one Gram-positive and three Gram-negative bacteria has been previously reported by Benhouda et al. (2014). Contrarily to the present work, the authors reported antimicrobial activity against *P. aeruginosa* and *P. mirabilis*, which can be due to different composition of the tested extracts, associated to differences of the extraction method, collection period, edaphoclimatic, and genetic factors, among others (Hansen & Wold, 2010).

4. Conclusion

This work aimed in the characterization of an edible wild plant for which almost no studies have been performed. For the first time, the nutritional composition of *U. rupestris* leaves was thoroughly evaluated, allowing to conclude that this plant can be included in a diet as a source of nutrients and bioactive compounds, such as omega-3 fatty acids, organic acids, vitamin E (tocopherols) and phenolic compounds, mainly flavonoids.

Table 5
Antimicrobial activity of the hydroethanolic and decoction extracts of *U. rupestris* (mean ± SD, n = 3).

Antibacterial activity	UR1 Hydroethanolic MIC	UR1 Hydroethanolic MBC	UR1 Decoction MIC	UR1 Decoction MBC	UR2 Hydroethanolic MIC	UR2 Hydroethanolic MBC	UR2 Decoction MIC	UR2 Decoction MBC	UR3 Hydroethanolic MIC	UR3 Hydroethanolic MBC	UR3 Decoction MIC	UR3 Decoction MBC	Ampicillin (20 mg/mL) MIC	Ampicillin (20 mg/mL) MBC	Imipenem (1 mg/mL) MIC	Imipenem (1 mg/mL) MBC	Vancomycin (1 mg/mL) MIC	Vancomycin (1 mg/mL) MBC
Gram-negative bacteria																		
Escherichia coli	20	> 20	10	20	20	> 20	20	> 20	20	> 20	5	> 20	< 0.15	< 0.15	< 0.078	< 0.078	nt	nt
Klebsiella pneumoniae	> 20	> 20	> 20	> 20	20	> 20	10	20	10	20	> 20	20	10	20	< 0.078	< 0.078	nt	nt
Morganella morganii	> 20	> 20	> 20	> 20	20	> 20	10	20	> 20	> 20	> 20	20	20	> 20	< 0.078	< 0.078	nt	nt
Proteus mirabilis	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	< 0.15	< 0.15	< 0.078	< 0.078	nt	nt
Pseudomonas aeruginosa	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	> 20	0.5	1	nt	nt
Gram-positive bacteria																		
Enterococcus faecalis	> 20	> 20	20	> 20	> 20	> 20	20	> 20	20	> 20	20	> 20	< 0.15	< 0.15	nt	nt	< 0.078	< 0.078
Listeria monocytogenes	> 20	> 20	10	> 20	5	20	5	20	20	> 20	20	20	< 0.15	< 0.15	< 0.0078	< 0.0078	nt	nt
MRSA	20	> 20	20	> 20	10	> 20	20	> 20	20	> 20	20	> 20	< 0.15	< 0.15	nt	nt	< 0.078	< 0.078

MRSA-Methicillin resistant *Staphylococcus aureus*; MIC-minimal inhibitory concentration; MBC-minimal bactericidal concentration; UR1, UR2 and UR3: samples from different geographic origins; nt-not tested.

Both the hydroethanolic and decoction extracts were able to promote the scavenging of free radicals, as well as to inhibit lipid peroxidation and prevent oxidative damage. In addition, they did not evidenced cytotoxicity in the assayed concentrations and were capable of inhibiting the growth of different Gram-positive and Gram-negative multi-resistant bacteria. Although several ethnobotanical studies describe the use of this plant for its anti-inflammatory properties, in the present study, both extracts did not evidence such activity in the assayed concentrations. This may be related to the selected assay/tested mechanism in the present study or even to the tested fraction/extract of the plant. In fact, considering that previous *in vivo* studies have supported the use of *U. rupestries* in traditional medicine for its anti-inflammatory properties, it would be worthwhile to further investigate the hydroethanolic extract using other methodologies (including *in vivo* assays) as well as testing different fractions containing other compounds such as polysaccharides.

In sum, the results herein reported are a contribution to enhance the preservation and knowledge on neglected and underexploited Mediterranean plant species, that once were seen as valuable food sources but whose consumption currently fell into disuse.

Declaration of Competing Interest

The authors declare that they do not have any conflict of interest.

Acknowledgements

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.05.139.

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