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Chemical composition and bioactive properties of the wild edible plant *Raphanus raphanistrum* L

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ABSTRACT

Recently, there has been an interest regarding the consumption of wild edible plants in modern diets. However, there is still scarce information about several wild vegetables traditionally consumed. Therefore, this work aims on documenting the nutritional and chemical composition of wild radish (*Raphanus raphanistrum* L.), as well as its bioactive potential. Results showed that wild radish is a potential source of beneficial compounds, including vitamin E, polyunsaturated fatty acid (particularly α-linolenic acid) and different phenolic compounds, in which fourteen phenolics were identified, with kaempferol-3,7-*O*-di-rhamnoside being the most abundant. The bioactive potential was exploited using hydroethanolic and decoction extracts. Both proved to inhibit several Gram-positive and Gram-negative bacteria and revealed antioxidant activity, while cytotoxicity against non-tumor cell was not observed. In general, results evidence the interest in recovering the use of this wild vegetable as part of a varied diet, which can bring several health benefits.

1. Introduction

Since ancient times, wild edible plants are collected by humans to be used as a food source. Additionally, due to the presence of biologically active compounds, many of these plants, or their parts, are also used in traditional medicine (Ferreira, Morales, & Barros, 2016). Along the history, wild edible plants have played an important role, in particular during times of famine and scarcity. Nowadays, they are still being exploited on a daily basis in several developing countries to provide an adequate level of nutrition. In contrast, they are underutilized in modern societies, mainly due to life-style changes caused by industrialization and urbanization. Notwithstanding, because they are part of the cultural heritage of several regions in the world, they continue to be traditionally consumed by different communities, mainly in rural regions (Pinela, Carvalho, & Ferreira, 2017). In addition, more recently, there is a growing interest of consumers regarding several wild plants since their consumption is being promoted by some culinary chefs, more information about composition and associated health benefits is being disseminated and there is a rising concern about the quality of mass-produced crops. Also, the importance of these natural resources in food security has been increasingly emphasized worldwide (Łuczaj et al., 2012).

The wild edible plant *Raphanus raphanistrum* L., commonly designated as wild radish (or as "labrestos" or "saramagos" in Portuguese) (Bicho, 2015), belongs to the Brassicaceae family, being characterized as an erect herb, with trichomes distributed along the plant, pubescent and alternated leaves and actinomorphic flowers, clearly veined, with coloration ranging from white to violet (Scalon & Souza, 2002). This specie is native from Europe, being mostly found in the southern Europe and all over the Mediterranean region. Because it is distributed in temperate and subtropical climate regions, its habitat is extremely vast, being also frequently found in the southeast of United States of America and southern Australia (Scalon & Souza, 2002). Among the different parts of the plant that may have edible use (leaves, young stems, flowers, roots and seeds), the leaves are the most frequently consumed. These have a slightly spicy taste and can be eaten either raw in salads or cooked in soups and as boiled vegetables (Maldini et al., 2017).

So far, there is a lack of information about this wild vegetable, with the existing data referring only to its composition in glucosinolates (Maldini et al., 2017). Therefore, in this study, the leaves of wild radish were thoroughly characterized in terms of its nutritional and chemical composition, giving special attention to the bioactive properties of hydroalcoholic and decoctions extracts, aiming to valorize *R. raphanistrum* as a traditionally used wild vegetable that can be included in

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modern diets.

2. Materials and methods

2.1. Samples

Leaves in a mature stage of *Raphanus raphanistrum* L. were collected during October 2017 from plants growing wild in Vila Real, Portugal (41° 17' 34" N, 7° 47' 16" W, 614 m a.s.l.). Three lots of freshly harvested leaves (~100 g each one) were collected and lyophilized (FreeZone 4.5, Labconco, MO, USA). A specimen voucher was deposited in the herbarium of the School of Agriculture, Polytechnic Institute of Bragança (Portugal). The dry leaves were crushed to a fine powder (20 mesh) and stored in the best condition (protected from light and humidity) until analysis was performed.

2.2. Hydroalcoholic and decoction extracts

Hydroalcoholic extractions were performed by stirring the plant material (2.5 g) with 30 mL of ethanol/water (80:20, *v/v*) at 25 °C, under magnetic stirring for 1 h and filtered through Whatman No. 4 paper. The residue was then re-extracted with additional 30 mL of the hydroalcoholic mixture. The combined extracts were evaporated at 40 °C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and further lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA).

Decoctions were performed by adding 200 mL of distilled water to the sample (1 g), and boiled for 5 min (heating plate, VELP scientific). Subsequently, the mixtures were left to stand for 5 min and then filtered through Whatman No. 4 paper. The obtained decoctions were frozen and lyophilized.

2.3. Chemical parameters

2.3.1. Nutritional compounds and energetic value

Sample were analysed in terms of nutritional compounds (moisture, fat, ash, proteins and carbohydrates) according to the AOAC methods (AOAC, 2016). Briefly, moisture was determined by drying fresh leaves at 105 ± 5 °C until constant weight. Crude protein was evaluated by macro-Kjeldahl method ($N \times 6.25$) using an automatic distillation and titration unit (model Pro-Nitro-A, JP Selecta, Barcelona) (AOAC 978.04), ash content was determined by incineration at 600 ± 15 °C (AOAC 923.03), and the crude fat was determined by extraction with petroleum ether using a Soxhlet apparatus (AOAC 920.85). Total carbohydrates were determined by difference according to the equation:

Total carbohydrates (g/100 g fw) = $100 - (g_{moisture} + g_{fat} + g_{ash} + g_{proteins})$.

Energy was determined according to the Atwater system following the equation:

(kcal/100 g fw) = $4 \times (g_{proteins} + g_{carbohydrates}) + 9 \times (g_{fat})$.

2.3.2. Free sugars

Free sugars were determined in the lyophilized sample and in both extracts (hydroalcoholic and decoction), using melezitose (Matreya, PA, USA) as internal standard (IS). The lyophilized sample was extracted using a methodology previously described (Barros et al., 2013), while the hydroalcoholic and decoction extracts (30 mg) were re-dissolved in 2 mL of distilled water and filtered through a 0.22 µm disposable LC filter disk. The compounds separation was performed using a high performance liquid chromatography system coupled with a refraction index detector (HPLC-RI, Knauer, Smartline system 1000, Berlin, Germany), using a Eurospher 100–5 NH2 column (4.6 × 250 mm, 5 mm, Knauer), operating at 35 °C (7971 R Grace oven) for chromatographic separation as previously described by Barros et al. (2013). Compounds were identified by comparison with standards (D(−)-fructose:, D(+)-sucrose, D(+)-glucose, D(+)-trehalose and D(+)-raffinose pentahydrate, Sigma-Aldrich (St. Louis, MO, USA), and quantification (calibration curves 24–0.625 mg/mL) was performed by the IS method. Results were processed using the Clarity 2.4 software (DataApex, Prague, Czech Republic) and expressed in g per 100 g of fresh weight (fw) or in mg per g extract, for plant or the extracts, respectively.

2.3.3. Organic acids

Organic acids were determined in the lyophilized sample and in both extracts (hydroalcoholic and decoction). The lyophilized sample was extracted using a methodology previously described and optimized (Barros et al., 2013) while the extracts (10 mg), were re-dissolved in 1 mL of metaphosphoric acid (4.5%) and filtered through a 0.22 µm disposable LC filter disk. The analysis was performed by ultra-fast liquid chromatography coupled with a diode-array detector (UFLC-PDA, Shimadzu Corporation, Japan) as previously described (Barros et al., 2013). Compounds were identified and quantified (calibration curves ranging from 10 to 0.008 mg/mL for oxalic, quinic, malic, ascorbic, citric, while fumaric acid 400–3125 µg/mL) by comparison of the retention time, spectra and peak area recorded at 245 nm and 215 nm (for ascorbic acid and remaining acids, respectively), with those obtained from commercial standards (oxalic, quinic, malic, ascorbic, citric and fumaric acids, Sigma-Aldrich, St. Louis, MO, USA). The results were recorded and processed using LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan), and were expressed in g/100 g fw or in mg per g extract, for plant or the extracts, respectively.

2.3.4. Fatty acids

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID), after the extraction and derivatization procedures previously described by Obodai et al. (2017). The analysis was carried out with a DANI model GC 1000 instrument equipped with a *split/splitless* injector, 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 oven temperature program was as follows: the initial temperature of the column was 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. The carrier gas (hydrogen) operated at a constant flow-rate of 1.1 mL/min, measured at 100 °C. *Split* injection (1:50) was carried out at 250 °C. Fatty acids identification and quantification (Clarity DataApex 4.0 Software, Prague, Czech Republic) were performed by comparing the relative retention times of fatty acid methyl ester (FAME) peaks from samples with standards (reference standard mixture 47,885-U, Sigma, St. Louis, MO, USA). Results were expressed in mg per 100 g fw of each detected fatty acid.

2.3.5. Tocopherols

Tocopherols were determined in the lyophilized sample and in both extracts (hydroalcoholic and decoction). The lyophilized sample was extracted as previously described (Barros, Carvalho, Morais, & Ferreira, 2010) while the extracts (40 mg), were re-dissolved in 2 mL of hexane and filtered through a 0.22 µm disposable LC filter disk. Separation, identification and quantification of the compounds were performed using a HPLC system coupled to a fluorescence detector (FP-2020; Jasco, Easton, USA) programmed for excitation at 290 nm and emission at 330 nm (Barros et al., 2013) using tocol (Matreya, Pleasant Gap, PA, USA) as internal standard. Tocopherols standards (α-, β-, γ-, and δ-isoforms, Sigma-Aldrch, St. Louis, MO, USA) were used for compounds identification and quantification (0.075 to 8 µg/mL) by the internal standard method. The results were processed using the Clarity 2.4 software (DataApex, Prague) and expressed in mg/100 g fw or mg per g extract, for sample and extracts, respectively.

2.3.6. Phenolic compounds

The extracts were re-dissolved in methanol/water (80:20, *v/v*, 5 mg/mL) and filtered through a 0.22 µm disposable LC filter disk.

Phenolic compounds were evaluated using a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) system equipped with a diode array detector (280, 330 and 370 nm as preferred wavelengths) coupled to an electrospray ionization mass spectrometry detector (MS), operating under the conditions described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). Chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (3 µm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column thermostatted at 35 °C. The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. MS detection was performed in negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source. Nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage of 5 kV, a source temperature of 325 °C, a capillary voltage of −20 V. The tube lens offset was kept at a voltage of −66 V. The full scan covered the mass range from *m/z* 100 to 1500. The collision energy used was 35 (arbitrary units). Data acquisition was carried out with Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

The identification was made by comparison of retention times, UV-VIS and mass spectra of the sample compounds with those obtained from the available standards, as also with reported data from literature, and tentatively identified by using the fragmentation pattern. The phenolic compound estimation was made using the calibration curves obtained from standards (200–5 µg/mL; *p*-coumaric acid, ferulic acid and quercetin-3-*O*-glucoside, Extrasynthese, Genay, France), which were constructed based on the UV–Vis signal (280, 330, and 370 nm). A manual integration using baseline to valley integration mode with baseline projection was performed to obtained the area of the peaks. The results were expressed in mg/g extract.

2.4. Bioactive properties

2.4.1. Evaluation of in vitro antioxidant properties

The extracts were re-dissolved in ethanol:water (80:20, *v/v*) and water for hydroalcoholic and decoctions extracts, respectively, at a final concentration of 5 mg/mL and further diluted at different concentrations (in the range of 5–0.0391 mg/mL) to perform the distinct in vitro assays. The antioxidant potential was evaluated by the DPPH radical-scavenging activity, reducing power, β-carotene bleaching inhibition and lipid peroxidation inhibition (LPI) assays, as previously described (Sarmento, Barros, Fernandes, Carvalho, & Ferreira, 2015).

2.4.1.1. DPPH radical scavenging activity. This methodology was performed using an ELX800 microplate reader (Bio-Tek Instruments, Inc.). Different concentrations of the extracts (30 µL) and methanolic solution (270 µL) containing *2,2*-diphenyl-1-picrylhydrazyl radicals (DPPH, 6×10^{-5} mol/L, Alfa Aesar, Ward Hill, MA, USA) were mixed and left to stand in the dark for 30 min. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm.

2.4.1.2. Reducing power. 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 g/100 mL, 0.5 mL). For each concentration, the mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10 g/100 mL, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionised water (0.8 mL) and ferric chloride (0.1 g/100 mL, 0.16 mL), and the absorbance was measured at 690 nm.

2.4.1.3. Inhibition of β-carotene bleaching. Was assessed through the β-carotene/linoleate assay, measured at 470 nm, and determined using the following equation:

(*β* − carotene absorbance after2 hof assay/initial absorbance) × 100.

2.4.1.4. TBARS (thiobarbituric acid reactive substances) assay. Was assessed in porcine brain homogenates by measuring the decreasing of thiobarbituric acid reactive substances (TBARS) at the absorbance of 532 nm. Lipid peroxidation inhibition (%) was calculated using the following equation:

[(A × B)/A] × 100 %.where A and B were the absorbance of the control and the sample solution, respectively.

The results of the assays were expressed as EC_{50}, corresponding to the extract concentrations providing 50% of antioxidant activity, calculated from the graphs of antioxidant activity percentages (DPPH assay, β-Carotene bleaching inhibition and lipid peroxidation inhibition) against extract concentrations or 0.5 of absorbance at 690 nm in the case of the reducing power assay. Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard.

2.4.2. Antimicrobial activity

Antimicrobial activity was evaluated using the broth microdilution method according to Pires et al. (2018). The microorganisms used were clinical isolates and included three Gram-positive bacteria (*Enterococcus faecalis*, *Listeria monocytogenes* and *methicillin-resistant Staphylococcus aureus*) and five Gram-negative bacteria (*Escherichia coli*, *Klebisiella pneumoniae*, *Morganela morganii*, *Proteus mirabilis* and *Pseudomonas aeruginosa*). The minimum inhibitory concentration (MIC) was determined for the hydroalcoholic and decoction extracts and evaluated through the colorimetric microbial viability based on the reduction of iodonitrotetrazolium chloride (0.2 mg/mL). The minimum bactericidal concentration (MBC) was evaluated by plating the content of the microwells that did not present coloration in the MIC assay. Different antibiotics were used as negative control, namely ampicillin and imipenem for Gram-negative bacteria, and vancomycin and ampicillin for Gram-positive bacteria. A positive control was prepared using culture broth Muller Hinton Broth (MHB) or Tryptic Soy Broth (THB) plus 5% of dimethyl sulfoxide (DMSO) inoculated with each tested bacteria.

2.4.3. Hepatotoxic activity

Was evaluated following the method described by Abreu et al. (2011), using a primary cell culture (PLP2) prepared from a porcine liver and different concentrations of the hydroalcoholic or decoction extracts, ranging from 400 µg/mL to 6.5 µg/mL. The results were measured through the Sulforhodamine B method and results were expressed as GI_{50} values (concentration that inhibits 50% of cell growth). Ellipticine was used as positive control.

2.5. Statistical analysis

For each analysis three samples were used and all assays were performed in triplicate. The results were expressed as mean ± standard deviation (DP). The results were analysed by a 1-variance analysis (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$. In case there were less than three sample groups, the results were analysed using a Student's *t*-test. The statistical treatment was performed by the SPSS v. 23.0.

3. Results and discussion

3.1. Chemical characterization of R. raphanistrum

The results of the nutritional value, free sugars and organic acids composition are shown in Table 1. Carbohydrates were the most abundant nutritional compound, followed by proteins, ash and lipids. This is in agreement with the nutritional compounds content reported by Kala and Prakash (2006) for a *R. raphanistrum* sample from India. However, a higher amount of moisture (94.60 g/100 g fw), and lower content of protein, ash and lipids (0.55, 0.55 and 0.06 g/100 g fw, respectively) were reported in comparison with the plant studied in this work. This may occur due to the different climatic and soil conditions

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Table 1
Nutritional value, energetic value, free sugars and organic acids of the studied plant *R. raphanistrum* (mean ± SD).

Nutritional value	
Moisture	89.9 ± 0.6
Ash (g/100 g fw)	1.58 ± 0.08
Proteins (g/100 g fw)	4.04 ± 0.01
Lipids (g/100 g fw)	0.23 ± 0.03
Carbohydrates (g/100 g fw)	4.22 ± 0.08
Energy (kcal/100 g fw)	35.1 ± 0.1
Free sugars (g/100 g fw)	
Fructose	0.153 ± 0.004
Glucose	0.348 ± 0.003
Trehalose	0.034 ± 0.001
Total free sugars	0.53 ± 0.01
Organic acids (g/100 g fw)	
Oxalic acid	0.706 ± 0.001
Quinic acid	0.62 ± 0.01
Malic acid	0.58 ± 0.01
Ascorbic acid	0.008 ± 0.001
Citric acid	0.613 ± 0.005
Fumaric acid	0.005 ± 0.001
Total organic acids	2.54 ± 0.01

Free sugars calibration curves: fructose ($y = 1.04\times$, $R^2 = 0.999$; LOD = 0.05 mg/mL; LOQ = 0.18 mg/mL), glucose ($y = 0.935\times$, $R^2 = 0.999$; LOD = 0.08 mg/mL; LOQ = 0.25 mg/mL) and trehalose ($y = 0.991\times$, $R^2 = 0.999$; LOD = 0.07 mg/mL, LOQ = 0.24 mg/mL).
Organic acids calibration curves: oxalic acid ($y = 9 \times 10^6$ x + 377,946; $R^2 = 0.994$; LOD = 12.55 μg/mL; LOQ = 41.82 μg/mL); quinic acid ($y = 612{,}327\times + 16{,}563$; $R^2 = 1$; LOD = 24.18 μg/mL; LOQ = 80.61 μg/mL); malic acid ($y = 863{,}548\times + 55{,}591$; $R^2 = 0.999$; LOD = 35.76 μg/mL; LOQ = 119.18 μg/mL); ascorbic acid ($y = 7E{+}07\times + 60{,}489$; $R^2 = 0{,}999$; LOD = 367 μg/mL; LOQ = 1222 μg/mL); citric acid ($y = 1 \times 10^6 x + 16{,}276$; $R^2 = 1$; LOD = 10.47 μg/mL; LOQ = 34.91 μg/mL) and fumaric acid ($y = 148{,}083\times + 96{,}092$; $R^2 = 1$; LOD = 0.08 μg/mL; LOQ = 0.26 μg/mL).

Table 2
Composition of fatty acids and tocopherols (mg/100 g fw) of *R. raphanistrum* leaves (mean ± SD).

Fatty acids	
C6:0	0.14 ± 0.02
C8:0	0.13 ± 0.02
C10:0	0.068 ± 0.006
C12:0	0.27 ± 0.02
C14:0	0.44 ± 0.05
C15:0	0.44 ± 0.04
C16:0	25.2 ± 2.3
C16:1	0.31 ± 0.03
C17:0	0.40 ± 0.03
C18:0	3.6 ± 0.3
C18:1n9	2.4 ± 0.3
C18:2n6	24 ± 2
C18:3n3	171 ± 16
C20:0	0.52 ± 0.04
C20:2	0.32 ± 0.03
C20:3n3 + C21:0	0.71 ± 0.07
C20:5n3	0.14 ± 0.01
C22:0	1.07 ± 0.08
C22:1n9	0.87 ± 0.09
C24:0	0.51 ± 0.06
C24:1	0.79 ± 0.09
SFA	33 ± 3
MUFA	4.4 ± 0.5
PUFA	196 ± 18
Tocopherols	
α-Tocopherol	0.89 ± 0.01
β-Tocopherol	0.041 ± 0.002
γ-Tocopherol	0.084 ± 0.003
δ-Tocopherol	0.052 ± 0.001
Total tocopherols	1.07 ± 0.01

C6:0 – Caproic acid; C8:0 – Caprylic acid; C10:0 – Capric acid; C12:0 – Lauric acid; C14:0 – Myristic acid; C15:0 – Pentadecanoic acid; C16:0 – Palmitic acid; C16:1 – Palmitoleic acid; C17:0 – Heptadecanoic acid; C18:0 – Stearic acid; C18:1n9 – Oleic acid; C18:2n6 – Linoleic acid; C18:3n3 – Linolenic acid; C20:0 – Arachidic acid; C20:2 – *cis*-11,14 – Eicosadienoic acid; C20:3n3 + C21:0 – Eicosatrienoic acid and Heneicosanoic acid; C20:5n3 – Eicosapentaenoic acid; C22:0 – Behenic acid; C22:1n9 – Erucic acid; C24:0 – Lignoceric acid; C24:1 – Nervonic acid. SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.
Tocopherols calibration curves: α-tocopherol ($y = 1.295\times$; $R^2 = 0.991$; LOD: 18.06 ng/mL, LOQ: 60.20 ng/mL); β-tocopherol ($y = 0.396\times$; $R^2 = 0.992$; LOD: 25.82 ng/mL, LOQ: 86.07 ng/mL); γ-tocopherol ($y = 0.567\times$; $R^2 = 0.991$; LOD: 14.79 ng/mL, LOQ: 49.32 ng/mL); δ-tocopherol ($y = 0.678\times$; $R^2 = 0.992$; LOD: 20.09 ng/mL, LOQ: 66.95 ng/mL).

(de Oliveira et al., 2016). Although, a comparable content of total carbohydrates was found for both Indian and Portuguese samples (4.22 vs 4.23 g/100 g fw) (Kala & Prakash, 2006).

Comparing the obtained results with those reported for commonly consumed species belonging to the Brassicaceae family (*Brassica oleracea* var. *acephala* (cabbage), *Brassica juncea* (mustard greens), *Raphanus sativus* (red radish)), one can observe that all presented a very high content of moisture, with *R. raphanistrum* presenting the most abundant content in protein and lowest lipid contents (Goyeneche et al., 2015; NEPA, 2011; USDA, 2016). Compared to *R. raphanistrum* leaves, *R. sativus* was the one presenting the most identical nutritional profile (crude protein 3.81 g/100 g fw, ash 1.70 g/100 g fw, total carbohydrates 4.04 g/100 g fw and lipids 0.37 g/100 g fw) (Goyeneche et al., 2015). This is most probably related to the fact that both these species belong to the same genus.

The energetic value calculated for the leaves of *R. raphanistrum* was considerably low, which makes this plant suitable for low carb diets, simultaneous allowing a higher diversification of the consumed food (Morales et al., 2012).

R. raphanistrum presented simultaneously three free sugars, namely the disaccharide trehalose and two monosaccharides, glucose and fructose. The predominant compound was glucose, the most prevalent sugar in nature, followed by fructose and smaller amounts of trehalose (Table 1). Organic acids are known to affect some characteristics of vegetables, such as flavour, aroma and appearance. It has been reported that malic acid has a strong permanent taste, which is not immediately perceived, while citric acid has pronounced and momentary flavour, which resembles lemon (Food Ingredients Brasil, 2015). Besides affecting the organoleptic characteristics of foods, organic acids can also interfere in the microbial stability, nutritional value and consumers acceptability, being frequently used in the food industry as acidifying compounds (Santos, 2013). In this work, six organic acids were identified in *R. raphanistrum* leaves (Table 1), with oxalic acid being the predominant compound, followed by quinic, citric and malic acids. It is known that vegetables with high concentration of oxalic acid, such as *S. oleracea* (0.97 g/100 g), *Beta vulgaris* L. (beet leaves, 0.61 g/100 g) or *Petroselinum crispum* (Mill.) Nym. (parsley, 1.7 g/100 g) leaves (USDA, 2016) should be consumed moderately, because the high intake of oxalates may promote kidney stones, intestinal mucosa irritation and also interferes in calcium absorption (Benevides, de, Souza, Souza, & Lopes, 2011; Vega & Florentino, 2000). Although the content of oxalic acid present in *R. raphanistrum* (0.706 g/100 g fw) was lower than that of spinach and parsley, it is reasonably high, being greater than that found in several vegetables, including red radish leaves (0.48 g/100 g). Therefore, its consumption should be avoided by individuals prone to

develop diseases such as kidney stones, arthritis, gout and rheumatism (Benevides et al., 2011).

Table 2 presents the fatty acid profile of *R. raphanistrum* leaves, showing that polyunsaturated fatty acids (PUFA) was the major group, followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). A total of 22 fatty acids were identified in the wild radish leaves, representing, to the best of our knowledge, the first report on the fatty acid composition of this species Wild radish leaves presented a predominant composition of unsaturated fatty acids, particularly omega-3 (ω-3) PUFA, due to the high content of α-linolenic acid (C18:3n3; 171 mg/100 g fw). Similar results were also observed in several other wild edible green vegetables, such as *Beta maritima* L., *Rumex papillaris* Boiss. & Reut., *Rumex pulcher* L. e *Taraxacum obovatum* (Willd.) DC. (Morales et al., 2012). *R. raphanistrum* leaves also revealed considerable values of palmitic (C16:0; 25.2 mg/100 g fw) and linoleic (C18:2n6; 24 mg/100 g fw) acids (Table 2).

Several studies have suggested that the consumption of food products of vegetable origin with high levels of α-linolenic acid (ω-3) can reduce the risk of cardiovascular diseases, in particular when SFA are replaced by PUFA in a rate between 5 and 10% of the consumed energy (Lira, Barros Silva, Figueirêdo, & Bragagnolo, 2014; Santos et al., 2013).

In addition, it has been reported that the ratio of ω-6/ω-3 levels should be lower than 4.0, since an unbalanced ratio due to the predominance of ω-6 has been associated with prothrombotic and pro-inflammatory effects (Simopoulos, 2016). In this sense, the inclusion of *R. raphanistrum* in the diet, and its increased consumption, can be beneficial to health due to its interesting fatty acid profile (Table 2).

Vitamin E is constituted by four isoforms of α-, β-, γ- and δ-tocopherols and α-, β-, γ- and δ-tocotrienols, which have been associated with a variable of human health effects, acting as anti-inflammatory, antioxidants and neuroprotectors (Singh, Beattie, & Seed, 2013). All the four isoforms of tocopherols were identified and quantified in the leaves of *R. raphanistrum*, with predominance of α-tocopherol as show in Table 2.

The dietary reference intake for vitamin E (expressed only in the form of α-tocopherol) provided by the Food and Nutrition Board of the Institute of Medicine, varies with ages, being the Recommended Dietary Allowances set between 6 mg for 1 to 3 years old children to 19 mg/day for breastfeeding women (Monsen, 2000; Institute of Medicine, 2000). Therefore, the consumption of wild radish leaves as a green vegetable in a varied and balanced diet contributes to attaining an adequate daily intake level of vitamin E (1.07 mg/100 g fw, Table 2).

3.2. Chemical characterization of *R. raphanistrum* extracts

Besides the chemical characterization of wild radish leaves, both prepared extracts (hydroalcoholic and decoction) were also evaluated regarding their composition in free sugars, organic acids, tocopherols and phenolic compounds. The analysis of these molecules in aqueous extracts is of great relevance, since it allows to determine the loss of compounds after a cooking process.

In both extracts, the main identified sugars were glucose and fructose. In comparison to the raw plant profile (Table 1), only trehalose was not identified in the extracts, possibly due to the low concentration of this sugar in the plant. A significantly higher total free sugars content was observed for the hydroalcoholic extract ($p < 0.001$) as show in Table 3, which may be due to factors described by Stalikas (2007), such as agitation and time of contact of the sample with the solvent.

Regarding the organic acids profile, both qualitative and quantitative differences were observed between the two extracts (Table 3). In contrast to total free sugars, a significantly higher amount of organic acids was found in the decoction extract (435 mg/g decoction extract vs. 344 mg/g hydroalcoholic extract) (Table 3). This significant difference can be explained by the greater solubility of these compounds in water extract then in less polar extraction systems (Daneshfar et al., 2012). Both extracts presented oxalic acid as predominant compound, however malic and citric acids were also present only in the decoction extract. The mixture of ethanol with water leads to the reduction of polarity, therefore leading to a decrease in the extraction of these compounds (Yuan, Leng, Shao, Huang, & Shan, 2014). By the contrary, ascorbic acid was additionally present in the hydroalcoholic extract, while being only found in trace amounts in the decoction extract. This is most probably due to the use of high temperature during the decoction extraction process (Santos, 2013).

Table 3
Free sugars (mg/100 g extract), organic acids (mg/100 g extract) and tocopherols (mg/100 g extract) of *R. raphanistrum* extracts (mean ± SD).

	Decoction	Hydroalcoholic	*t*-Students test
			p-value
Free sugars			
Fructose	27.8 ± 0.3	41.4 ± 0.7	< 0.001
Glucose	45.5 ± 0.6	65.5 ± 0.4	< 0.001
Total free sugars	73.3 ± 0.9	107 ± 1	< 0.001
Organic acids			
Oxalic	283 ± 2	344 ± 3	< 0.001
Malic	55.9 ± 0.3	nd	–
Ascorbic	tr	0.13 ± 0.001	–
Citric	96 ± 2	nd	–
Fumaric	tr	tr	–
Total organic acids	435 ± 4	344 ± 3	< 0.001
Tocopherols			
α-Tocopherol	nd	99 ± 1	–
Total tocopherols	nd	99 ± 1	–

nd – not detected (below LOD values); tr – traces (below LOQ values).

Concerning the tocopherols content, only α-tocopherol was detected, being present uniquely in the hydroalcoholic extract (Table 3). The absence of this lipophilic compound in the decoction extract was expectable, mainly due to its non-solubility in aqueous solvents, but also due to the high temperature applied during the decoction extraction.

The analysis of phenolic compounds profile in *R. raphanistrum* extracts were performed by HPLC-DAD-ESI/MS^n (Fig. 1). 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 4. Fourteen phenolic compounds were identified, comprising two hydroxycinnamoylquinic acid derivatives (ferulic and *p*-coumaric acid derivatives) and twelve flavonols derivatives (quercetin and kaempferol glycoside derivatives).

In Table 4, peak 1 ($[M-H]^-$ at *m/z* 337) was identified as 3-*p*-coumaroylquinic, yielding the base peak at *m/z* 191 [quinic acid-H]$^-$ and also gave an ion at *m/z* 173 [coumaric acid-H]$^-$ with an intensity 43% of the base peak. Similar fragmentation pattern was already reported by Clifford, Johnston, Knight, and Kuhnert (2003) and Clifford, Knight, and Kuhnert (2005) and used to distinguish 3-acylcoumaric acids. Peak 2 ($[M-H]^-$ at *m/z* 367) was identified as 3-*O*-feruloylquinic acid taking into account the same hierarchical keys previously developed by Clifford et al., (2003) and (2005). Both these compounds, as far as the authors knowledge were not reported previously in *R. raphanistrum*.

The following compounds were identified as quercetin (λ_{max} around 353 nm, and an MS^2 fragment at *m/z* 301) and kaempferol (λ_{max} around 346 nm, and an MS^2 fragment at *m/z* 285) glycoside derivatives. Peaks 3 and 4 presented the same pseudomolecular ion $[M-H]^-$ at *m/z* 609, revealing the alternative loss of hexosyl (*m/z* at 447; −162 u) and deoxyhexosyl (*m/z* at 301; −146 u) residues, indicating location of each residue on different positions of the aglycone. These compounds were tentatively identified as quercetin-*O*-deoxyhexoside-*O*-hexoside isomers. Thus, similar compounds have been previously described in a 70% ethanolic extract of *R. raphanistrum* aerial parts collected from the Giza field, Egypt (Ibrahim et al., 2016), therefore similar assumption

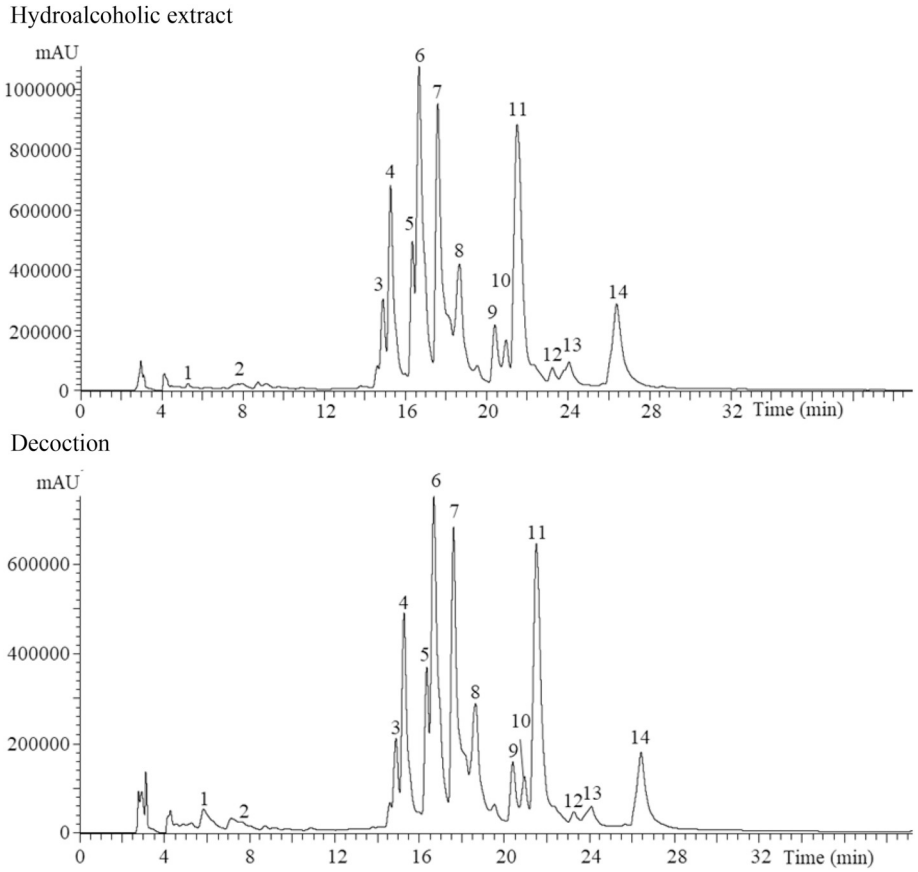

Fig. 1. Phenolic profile of the hydroalcoholic and decoction extracts of *Raphanus raphanistrum* recorded at 370 nm. The numbers presented in the chromatograms correspond to the compounds identified in Table 4.

Table 4
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, tentative identification, and a quantification estimation (mean ± SD) of the tentatively identified phenolic compounds in decoction and hydroalcoholic extracts of *R. raphanistrum*.

Peak	Rt	λmax	$[M-H]^-$	MS^2	Tentative identification	Decoction	Hydroalcoholic	*t*-Students test
	min)	(nm)	(*m/z*)	(*m/z*)		(mg/g extract)	(mg/g extract)	*p*-value
1	6.41	313	337	191(100),173(42),163(5),147(3),129(2)	3-*p*-Coumaroylquinic acid[a]	0.52 ± 0.01	0.16 ± 0.004	< 0.001
2	7.76	292,318	367	193(29),191(100),173(5),147(5),129(2)	3-*O*-Feruloylquinic acid[a]	0.22 ± 0.001	0.175 ± 0.001	< 0.001
3	14.88	255,353	609	463(60),447(100),301(15)	Quercetin-3-*O*-rhamnoside-7-*O*-glucoside[b]	0.94 ± 0.02	1.2 ± 0.03	< 0.001
4	15.25	254,353	609	463(49),447(100),301(15)	Quercetin-*O*-rhamnoside-*O*-glucoside[b]	2.34 ± 0.001	3.63 ± 0.01	< 0.001
5	16.31	263,346	771	285(100),257(20),151(13)	Kaempferol-*O*-tri-hexoside[c]	1.53 ± 0.01	1.88 ± 0.005	< 0.001
6	16.65	264,348	593	447(100),285(5)	Kaempferol-3-*O*-rhamnoside-7-*O*-glucoside[b,c]	4.07 ± 0.1	6 ± 0.2	< 0.001
7	17.58	263,346	593	447(100),431(47),285(8)	Kaempferol-*O*-rhamnoside-*O*-glucoside[b,c]	3.62 ± 0.06	4.47 ± 0.02	< 0.001
8	18.64	263,347	563	417(100),285(50),257(5),151(3)	Kaempferol-*O*-deoxyhexoside-*O*-pentoside[c]	1.8 ± 0.1	2.4 ± 0.1	0.001
9	20.39	263,348	739	593(100),431(20),285(12)	Kaempferol-3-*O*-(2″-glucosyl)-rhamnoside-7-*O*-rhamnoside[b]	1.04 ± 0.004	1.44 ± 0.01	< 0.001
10	20.90	261,326	931	785(100),609(18),285(5)	Kaempferol-3-*O*-feruloyldihexoside-7-*O*-rhamnoside[d]	0.85 ± 0.005	1.1 ± 0.03	< 0.001
11	21.48	263,346	577	431(100),285(11)	Kaempferol-3,7-*O*-di-rhamnoside[d]	4.85 ± 0.1	7.5 ± 0.1	< 0.001
12	23.19	261,330	917	771(5),609(100),285(11)	Kaempferol-3-*O*-caffeoyldihexoside-7-*O*-rhamnoside[d]	0.47 ± 0.02	0.7 ± 0.02	< 0.001
13	24.03	252,332	947	801(100),625(22),301(15)	Quercetin-3-*O*-feruloyldihexoside-7-*O*-rhamnoside[d]	0.68 ± 0.04	0.8 ± 0.02	0.002
14	26.37	262,330	931	785(100),609(11),285(5)	Kaempferol-*O*-feruloyldihexoside-*O*-rhamnoside[d]	1.84 ± 0.04	2.5 ± 0.02	< 0.001
					Total phenolic acids	0.74 ± 0.01	0.33 ± 0.01	< 0.001
					Total flavonoids	24.02 ± 0.4	33.26 ± 0.5	< 0.001
					Total phenolic compounds	24.76 ± 0.4	33.59 ± 0.5	< 0.001

Calibration curves: Peak 1: *p*-coumaric acid (y = 301,950× + 6966.7; R^2 = 0.999; LOD = 0.68 μg/mL; LOQ = 1.61 μg/mL); Peak 2- ferulic acid (y = 633,126× − 185,462; R^2 = 0.999; LOD = 0.20 μg/mL; 1.01 μg/mL); peaks 3 to 14: quercetin 3-*O*-glucoside (y = 34,843× − 160,173; R^2 = 0.9998; LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL). References applied for the tentative identification: a – Clifford et al. (2003), Clifford et al. (2005); b – Ibrahim et al. (2016), c –DAD and MS fragmentation pattern; d – Lin et al. (2014).

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Table 5
Antioxidant and hepatotoxicity activities of *R. raphanistrum* extracts (mean ± SD).

	R. raphanistrum		*t*-Students test
	Decoction	Hydroalcoholic	*p*-value
Antioxidant activity			
DPPH scavenging activity (EC_{50}[a]; mg/mL)	4.0 ± 0.1	3.12 ± 0.07	< 0.001
Ferricyanide/Prussian blue (EC_{50}[b]; mg/mL)	3.23 ± 0.03	1.01 ± 0.01	< 0.001
β-carotene/linoleate (EC_{50}[a]; mg/mL)	0.56 ± 0.02	0.26 ± 0.03	< 0.001
TBARS (EC_{50}[a]; mg/mL)	0.16 ± 0.06	0.108 ± 0.007	0.022
Hepatotoxicity (GI50 μg/mL)			
PLP2 (porcine liver primary culture)	> 400	> 400	–

EC_{50}: extract concentration corresponding to [a] 50% of antioxidant activity or [b] 0.5 of absorbance in the reducing power assay. Trolox EC_{50} values: 41 μg/mL (reducing power), 42 μg/mL (DPPH scavenging activity), 18 μg/mL (β-carotene bleaching inhibition) and 23 μg/mL (TBARS inhibition). GI_{50} values correspond to the sample 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 (PLP2).

were taken into account for these compounds, being tentatively assigned as quercetin-3-*O*-rhamnoside-7-*O*-glucoside and quercetin-*O*-rhamnoside-*O*-glucoside, respectively. Similarly, and taking into account the mentioned assumptions, compounds 6 and 7 ($[M-H]^-$ at *m/z* 593) were tentatively identified as kaempferol-3-*O*-rhamnoside-7-*O*-glucoside and kaempferol-*O*-rhamnoside-*O*-glucoside, respectively. Compound 9 ($[M-H]^-$ at *m/z* 739) should correspond to a kaempferol derivative bearing two deoxyhexosyl (−146 u) and one hexosyl (−162 u) residues. A compound with similar mass characteristics, i.e., kaempferol-3-*O*-(2″-glucosyl)-rhamnoside-7-*O*-rhamnoside, was reported in *R. raphanistrum* by Ibrahim et al. (2016), thus this compound was tentatively identified as that molecule. Mass characteristics of compound 5 ($[M-H]^-$ at *m/z* 771) indicated that it also corresponds to a kaempferol derivative bearing three hexosyl residues (−486 u), the observation of only one MS^2 fragment suggested that the three sugars were linked together, suggesting that the sugars constituted a trisaccharide (tri-hexoside), thus information about the identity of the sugar moieties and location onto the aglycone could not be obtained, so, the compound was tentatively identified as kaempferol-*O*-tri-hexoside. Compound 8 ($[M-H]^-$ at *m/z* 563) presented two MS^2 fragments at *m/z* 417 (−146 u, deoxyhexosyl moiety) and 285 (−132 u, pentosyl moiety), indicating alternative losses of each sugar moieties, pointing to their location on different positions of the aglycone, thus being tentatively identified as kaempferol-*O*-deoxyhexoside-*O*-pentoside. To the best of our knowledge both these compounds was not previously identified in *R. raphanistrum*. Compound 11 ($[M-H]^-$ at *m/z* 577) showed a similar fragmentation pattern as peak 8, with the respective losses of two deoxyhexosyl moieties (−146 u), and presenting a typical fragmentation pattern as kaempferol. A compound with similar mass characteristics, kaempferol-3,7-*O*-di-rhamnoside, was previously reported in *Rorippa indica* (Linn.), a plant species from the same family (Lin et al., 2014), thus this assumption was tentatively performed for this compound.

The remaining compounds (10 and 12–14) were all identified as acylated flavonols due to the observation of a loss of a feruloyl residue (−176 u) or caffeoyl (−162 u), which is coherent with all the identities of the remaining compounds, as well as their late elution, since the presence of the hydroxycinnamoyl residue implies a decrease in polarity, as also to their lower maximum absorbance. All these peaks were previously identified by Lin et al. (2014) and the assumptions were also taken into account for the species studied herein. Compounds 10 and 14 ($[M-H]^-$ at *m/z* 931) and 13 ($[M-H]^-$ at *m/z* 947), all presented a similar fragmentation pattern, revealing tree MS^2 fragments representing the successive loss of a deoxyhexosyl (−146 u), feruloyl (−176 u) and di-hexosyl (−324 u) residues. Taking into account the similar fragmentation pattern mentioned by Lin et al. (2014), these compounds were identified as kaempferol-3-*O*-feruloyldihexoside-7-*O*-rhamnoside, kaempferol-*O*-feruloyldihexoside-*O*-rhamnoside, and quercetin-3-*O*-feruloyldihexoside-7-*O*-rhamnoside, respectively. Similarly, compound 12 ($[M-H]^-$ at *m/z* 917) also revealed the presence of three MS^2 fragments at *m/z* 771 (−146 u, loss of a deoxyhexosyl moiety), 609 (−162 u, loss of a caffeoyl moiety) and 285 [kaempferol-H]$^-$ (−324 u, loss of a di-hexosyl residue), thus being tentatively assigned to a similar compound, with the same DAD and MS characteristics, as kaempferol-3-*O*-caffeoyldihexoside-7-*O*-rhamnoside.

The hydroethanolic extract presented a higher concentration in the tentatively identified phenolic compounds, in which flavonols were present in higher abundance (97–99% of the total phenolic content), being kaempferol-3,7-*O*-di-rhamnoside the most abundant compound in both extracts. Although the extraction of phenolic compounds is superior in the hydroalcoholic extract the values obtained for the decoctions are also relevant, since this plant can be consumed after cooking. The results demonstrate that the water used in the decoction extract promotes the extraction of phenolic compounds, thus the non-use of the cooking water implies the loss of many of these compounds in particular of the phenolic acids, which are found in higher amounts in the decoction extract.

3.3. Bioactive properties of *R. raphanistrum* hydroalcoholic and decoction extracts

Edible wild plants are commonly used as foods, nonetheless several are also used as traditional herbal medicines (Özen, 2010). Besides being consumed raw or cooked, *R. raphanistrum* has also been associated with beneficial properties, being traditionally used in treatment of hepatobiliary affections, dyspeptic problems, mainly related with biliary dyskinesia and bronchial affections (Neto & Simões, 2016). Therefore, this study also comprised the evaluation of different biological properties, namely antioxidant, cytotoxic and antimicrobial activities.

Data regarding the antioxidant and hepatotoxicity activities are presented in Table 5. Four different antioxidant activity assays (DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and TBARS inhibition assays) were performed. For both extracts, the lowest EC_{50} values (highest antioxidant activity) were observed in the TBARS inhibition and β-Carotene bleaching inhibition assays. Additionally, results show that, for all the four methods assayed, the hydroalcoholic extract exhibited a higher antioxidant potential compared to the decoction. Nevertheless, this last extract still presents interesting results, evidencing that a substantial loss of antioxidants occurs into the boiling water when wild radish is cooked. Therefore, its consumption can be more beneficial in the form of soups than as boiled vegetables. The superior antioxidant activity obtained for the hydroalcoholic extract may be explained by its higher content of phenolic compounds and tocopherols.

The antioxidant activity determined by the DPPH method of an aqueous extract of *R. raphanistrum* prepared by maceration at room temperature, has been previously reported by Küçükboyaci, Güvenç, Turan, and Aydin (2012). In that work a higher EC_{50} value was reported (8.78 mg/mL), corresponding to a lower antioxidant activity compared to the plant herein studied, which could be related to the different extraction procedures applied in both studies, but also to different applied solvents, in the case of the hydroethanolic extract. The extraction methodology highly affects the extraction of compounds, as it visual in the present, were the hydroethanolic extract presented a higher bioactivity in comparison to the aqueous extract. Moreover, these

Table 6
Antimicrobial activity of the studied plant *R. raphanistrum* extracts (mean).

	Hydroalcoholic		Decoction		Ampicillin (20 mg/mL)		Imipenem (1 mg/mL)		Vancomycin (1 mg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria										
Escherichia coli	2.5	20	2.5	20	< 0.15	< 0.15	< 0.0078	< 0.0078	nt	nt
Klebsiella pneumoniae	10	20	10	20	10	20	< 0.0078	< 0.0078	nt	nt
Morganella morganii	10	20	10	20	20	> 20	< 0.0078	< 0.0078	nt	nt
Proteus mirabilis	> 20	> 20	> 20	> 20	< 0.15	< 0.15	< 0.0078	< 0.0078	nt	nt
Pseudomonas aeruginosa	> 20	> 20	> 20	> 20	> 20	> 20	0.5	1	nt	nt
Gram-positive bacteria										
Enterococcus faecalis	20	> 20	20	> 20	< 0.15	< 0.15	nt	nt	< 0.0078	< 0.0078
Listeria monocytogenes	5	20	5	20	< 0.15	< 0.15	nt	nt	nt	nt
MRSA	10	> 20	10	> 20	< 0.15	< 0.15	nt	nt	< 0.0078	< 0.0078

MRSA: Methicillin resistant *Staphylococcus aureus*; MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; nt: not tested.

differences could also be due to other factors, that are known to affect the chemical composition of plants, such as the timing of harvesting, geographic location/climate and the plant adaptation to the soil conditions (Sampaio, Edrada-Ebel, & Da Costa, 2016). Additionally, differences in plants genotype may be associated with chemical variability, therefore affecting its bioactivity.

Concerning the evaluation of in vitro cytotoxic activity in non-tumor cells, both extracts did not presented activity against porcine hepatic cells, in the tested concentrations (Table 5).

Regarding the antibacterial activity results, no differences were found between the two evaluated extracts, being both active against six of the eight tested bacteria (Table 6). Both extracts showed the lowest MIC values against Gram-positive bacteria, namely against *E. coli* (MIC = 2.5 mg/mL) and L. *monocytogenes* (MIC = 5 mg/mL). The extracts also exhibited bactericidal effect against these two bacteria, frequently associated to foodborne diseases, although higher concentrations were needed (MBC = 20 mg/mL) to achieve the growth inhibition. In the tested concentrations, both type of extracts were ineffective against the Gram-negative bacteria *P. mirabilis* and *P. aeruginosa*. While previous works have reported the antimicrobial activity of the crude juice or extracts of *R. sativus* (red radish) against a broad range of bacteria (Kaymak, Ozturk, Ercisli, & Guvenc, 2015), as far as we know, there is no previous data regarding the antimicrobial activity of wild radish.

4. Conclusion

An extensive and detailed study comprising the chemical characterization of *R. raphanistrum* leaves, including its composition in nutritional compounds, free sugars, organic acids, fatty acids, vitamin E (tocopherols) and phenolic compounds was performed. The obtained results evidenced that wild radish is a good source of several beneficial health compounds, including PUFA fatty acids, α-tocopherol, phenolic compounds. Most phenolic compounds found in its composition were reported for the first time and included mainly kaempferol derivatives, but also quercetin derivatives and two caffeic acid derivatives. In addition to presenting a low energetic value, wild radish also showed higher protein and lower lipid content when compared to other green vegetables belonging to Brassicaceae family.

Both hydroalcoholic and decoction extracts showed antioxidant and antibacterial activity, without evidencing cytotoxicity against non-tumoral liver cells. The extracts were able to inhibit a broad range of bacteria, including both Gram-positive and Gram-negative bacteria, with a higher effect being observed against the former.

Overall, the obtained results demonstrate that *R. raphanistrum* can be a sustainable source of nutrients and compounds with high bioactive potential, highlighting the interest of incorporating this edible wild plant as a vegetable in a diversified and healthy diet.

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