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Chemical composition and antibacterial activity of red murta (*Ugni molinae* Turcz.) seeds: an undervalued Chilean resource

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Abstract

Murta (*Ugni molinae* Turcz) is an endemic Chilean specie mostly used in medical, cosmetic, and food industries. However, during the industrial processing of murta fruits, the biomass containing the seeds is discarded as an industrial byproduct that does not find significant uses yet. This work is a first approach to valorize murta biomass through the identification and quantification of principal chemical constituents and exploring their antibacterial properties. The proximal analysis revealed that murta seeds exhibited significant content of raw fiber (64%), crude fat (14%), crude protein (12%), and low levels of ashes (1.5%) and minerals (0.04–0.23%). Dietary fiber was mainly composed of lignin, cellulose, pectin, and hemicellulose. Polyunsaturated fatty acids (89.0%), monounsaturated fatty acids (7.7%), and saturated fatty acids (3.3%) were the main constituents of seed oils. The arginine, asparagine, glutamic acid, and glycine were the primary protein constituent amino acids. Tannin fractions, total polyphenolic content, and oxygen radical absorbance capacity as antioxidant activity were measured. The chromatographic and mass spectrometric analysis (HPLC–MS/MS) confirmed the presence of several phenolic compounds like phenolic acids, flavonols, flavones, proanthocyanidins, and high molecular weight polyphenols. The murta seed extract showed high antibacterial activity against both Gram positive (*Staphylococcus aureus*, *Bacillus cereus*, and *Streptococcus pyogenes*) and Gram negative (*Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*) bacterial strains. Murta seeds could be considered as a new source of nutritional components and bioactive compounds for different nutraceutical and food applications.

Keywords Chemical characterization · Polyphenols · Antioxidant · Liquid chromatography · Antibacterial activity · Murta (*Ugni molinae T.*) seeds

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Introduction

Myrtleberry, also known as Chilean guava, murtilla, or murta (*Ugni molinae* Turcz), is a Myrtaceae specie from southern Chile. The Chilean indigenous ethnic Mapuche have historically appreciated this shrub due to its ethnopharmacological uses as anti-inflammatory and pain relieve [1, 2]. Furthermore, the murta fruit has a pleasant taste as well as a sweet and floral aroma [3].

Regarding the fruit peel color, there are three different murta genotypes: red, pink, and white. In fruit, there is an average number of seeds ranging from 12 to 19, depending on the ecotype and it is not associated with the fruit size [4]. Currently, murta fruits are used for the industrial preparation of liquors, cosmetic products, jam, and jellies. In the latter case, the high-quality pectin contained in murta pulp is a clear benefit [5]. However, as a consequence of the industrialization of murta products, large amounts of by-products, mainly consisting of fruit seeds, are generated. It could have a negative environmental impact. Hence, the development of value-added products from seeds could increase the grower's profit margins, for instance, through the commercialization of the seed press cake [6].

On the other hand, the seeds have been considered as an alternative source of fibers and proteins for human and animal consumption. Besides, seeds of many plants contain uncommon fatty acids and lipids, which are valuable for human nutrition and health care. In this sense, flax seeds, pumpkin seeds, chia seeds, sesame seeds, nuts, and black currant seeds are currently recommended for daily human intakes due to the nutritional value and health-promoting activity of their components [7].

According to previous studies, murta leaves and fruit extracts are rich in phenolic compounds with biological properties such as anti-inflammatory and analgesic effects. The human erythrocytes protection from oxidative damage, alpha-glucosidase/alpha-amylase inhibition, as well as antimicrobial properties, have been also reported [1].

Despite the growing use of murta fruit in the Chilean food industry, only scarce and non-systematic information regarding seed composition and their potential applications have been evaluated. This research deals with murta seed chemical analysis, and the evaluation of the phenolic extracts biological activity, in this sense, proximal analysis, total polyphenolic content and different tannin fractions were determined, and HPLC–MS/MS technique was used to identified polyphenolic compounds. The biological activity of seed extracts was assessed by the oxygen radical absorbance capacity (ORAC) test and evaluating the antibacterial activity against several bacterial strains associated with food contamination and wound healing.

Materials and methods

Materials

Red murta fruits (*U. molinae* T.) were obtained in the local market from Temuco city, Araucanía Region, Chile. The fruits (2 kg) were washed with distilled water and the skin was removed using 1 mm sieve. The seeds were isolated, washed with distilled water and dried at room temperature (25 °C). The sample was pulverized in a stone mill and passed through 0.5 mm sieve. Lipids were extracted by seed treatment with anhydrous ether in a Soxhlet apparatus [8]. The defatted murta seeds were stored at − 20 °C until analyses. The average weight of 100 seeds was 943 µg.

Proximate analysis

The methods from the Association of Official Analytical Chemists (AOAC) [9] were adopted to determine the levels of crude protein (AOAC 920.87), moisture (AOAC 925.26), ashes (AOAC 940.263), raw fiber (AOAC 9912.43) and Lipids (AOAC 933.05). Total soluble carbohydrate was determined by the phenol–sulphuric acid method [10] using glucose (α-D-glucopyranose) as standard. The energy value for murta seed by-products is also reported [11].

Chemical analysis

Fiber

The detergent analysis in terms of the content of neutral detergent fiber (NDF), soluble fibers (SF), and acid detergent fibers (AF) and lignin (ADL) was carried out according to the Van Soest method [12]. Murta seed sample was boiled with the NDF and ADF solutions, respectively. The solution resulting in NDF treatment was dialyzed against distilled water and further dried for estimating SF. The respective fiber fractions were determined gravimetrically after drying and incineration. For ADL, another ADF-fraction was hydrolyzed with 72% H_2SO_4 (v/v), then washed with distilled water and acetone and dried. The sample ash content and mass loss are determined. The hemicellulose content is calculated by the difference in NDF-ADF, and cellulose content by the difference in ADF-ADL. The quantification of galacturonic acids determined the pectin content in SF fraction by the *m*-hydroxybiphenyl method [13]. Pure galacturonic acid monohydrate (purity > 99%, Fluka Chemie AG., Buchs, Switzerland) standard was used.

Fatty acids composition

An aliquot (0.2 g) of seed sample was boiled with 4 N HCl for one hour, filtrated, washed with HPLC-grade water and

extracted twice with petroleum ether using a Soxhlet apparatus. For the determination of total lipids, the extracted seed oil was methylated using sodium methoxide in *n*-hexane, then mixed with sodium hydrosulphate and centrifuged. The supernatant fraction was analyzed by gas chromatography coupled to a flame ionization detector (GC/FID) (Hewlett Packard HP6890, Agilent, Santa Clara, CA, USA) using an HP 225 column and H_2 as the carrier gas. The inlet temperature was 250 °C, the oven temperature was 230 °C, and the detector temperature was 280 °C. The used column is qualified to separate fatty acid methyl esters between C4 and C22, using the GLC 462 Nu-check calibration standard. HPCHEM Stations software (Agilent, Santa Clara, CA, USA) allows the fatty acid quantification, which was expressed as a percent of total fatty acid methyl esters.

Protein and amino acids

For crude protein determination, nitrogen concentration was determined by the Kjeldahl method (AOAC, 1995). The amino acid analysis was performed on an Amino Acid Analyzer LC 3000 (Eppendorf/Biotronik) using ion-exchange chromatography with ninhydrin post-column derivatization. Calibration standards were purchased at Onken GmbH, Gründau, Germany.

Protein was hydrolyzed with 6 N HCl before the determination of acid (Asp, Glu), neutral (Thr, Ser, Pro, Hyp, Ala, Gly, Val, Ile, Leu, Tyr, Phe) and alkaline (Lys, His, Arg) amino acids. Due to their instability, before the acid hydrolysis, the sulfur-containing amino acids (Met, Cys) were oxidized to methionine sulfone and cysteic acid using performic acid. Since the neutral amino acid tryptophan degrades at those conditions, the sample was hydrolyzed with 4 N LiOH at 110 °C for 20 h under anaerobic conditions [14].

The protein efficiency ratio (PER) was calculated from the regression equation proposed [15]. The chemical protein score (CS) was estimated comparing the ratio of the restrictive amino acid (amino acids having the shortest supply, a_i) to the concentration of this amino acid in the standard protein (a_{is}) as recommended by FAO/WHO/UNU report [16].

The essential amino acid index (EAAI), defined as the geometric mean of the ratios of the essential amino acids in a protein to those of a standard, was estimated by the FAO/WHO/UNU [16]. The nutritional index (NI) was calculated using a reported formula [11]. The approximated biological quality (BQ) was calculated as previously described [17].

Vitamin C

Vitamin C was determined by a method based on the reaction of dehydroascorbic acid with dinitrophenylhydrazine [18]. To 200 µl of the seed extract, 300 µl trichloracetic acid was added and mixed vigorously in a vortex. The mixture was centrifugated and 300 µl of the upper layer was removed and combined with 100 µl of dinitrophenylhydrazine reagent. This mixture was heated at 60 °C for one hour. After cooling down in an ice bath, 400 µl of H_2SO_4 was added and mixed vigorously. After 20 min in the dark, the samples were measured spectrophotometrically at a wavelength of 520 nm.

Minerals

Mineral analyses were conducted according to the A.O.A.C. methodology [9]. Ash was carefully transferred into a flask using 2.5 ml of 25% (v/v) HCl solution and digested for 5 min. Afterward, the container was filled up with H_2O to a final volume of 25 ml. The sample was heated, filtered, and acidified with ultrapure HNO_3 to a final sample concentration of 2% (v/v). For measuring with inductively coupled plasma optical emission spectrometry (ICP-OES; Spectroflame, Spectro, Kleve, Germany), samples were twofold diluted with ultrapure water before injection. Calibration ranges for the elements were adjusted according to the expected concentrations. Calibration standards were purchased at Merck, Darmstadt, Germany (multielement standard Merck IV; As-, Mo-, Se-, and Ti-ICP standard) and Alfa Aesar, Karlsruhe, Germany (P-, S-, V-plasma standard).

Phytochemical analysis

Polyphenol extraction

Murta seeds powder (250 mg) were mixed with ethanol (2 ml, 95%), shaken for 24 h at room temperature (25 °C) and centrifuged for 10 min at 5000×*g*. Four independent seeds extractions were done, and supernatants were stored at − 20 °C for further analysis.

Polyphenolic content of extracts

Total phenolic content in seed extracts, for standardization purposes before bactericide assay, was determined by the Folin–Ciocalteau method, using gallic acid (3,4,5-trihydroxybenzoic acid) as a standard [19]. Quantification was carried out at 750 nm based on a standard curve of gallic acid, and the results are given as mg of gallic acid equivalent (GAE)/100 g of fresh seeds (FW as fresh weight).

ORAC assay

The ORAC procedure used an automated plate reader with 96 well plates [20]. The analysis was carried out in phosphate buffer (pH 7.4) and 37 °C. Peroxyl radicals were generated using 2,2´-azobis(2-amidino-propane) dihydrochloride, which was prepared fresh for each run. Fluorescein was used as the substrate. Fluorescence conditions were as

follows: excitation at 485 nm and emission at 520 nm. The standard curve was linear between 0 and 50 µM of Trolox (6-hydroxy-2,5,7,8 tetramethyl-chroman-2-carboxylic acid) equivalents (TE). The results are expressed as µmol of TE/100 g seed.

Condensed tannin (CT)

The butanol/HCl/Fe^{3+} method for condensed tannin determination was used [21]. First, 0.5 ml of extract was mixed with 2 ml of butanol-containing reagent (51.5% acetone/43% butanol/5% 12 N concentrated HCl/0.5% H_2O) and 67 µl of Fe-reagent (2% w/v $FeNH_4(SO_4)_2$ in 2 N HCl). The final assay mixture, containing both sample and assay solutions in a 2.5 ml volume, was thus comprised (v/v) of 50.1% acetone, 33.5% butanol, 3.9% 12 N concentrated HCl, 7% dH_2O, 2.9% MeOH, and 2.6% Fe-reagent in a total volume of 2.5 ml. Aliquots (200 µL) of the mixture were removed and used as non-heated controls. Samples were heated to 70 °C for 2.5 h, allowed to cool to room temperature (20 °C), and the absorbance was read at 550 nm using a Victor™ X5 Multi-label plate reader (Perkin Elmer Inc.). For CT concentration determination, absorbances from unheated aliquots were subtracted from heated samples.

Ellagitannins (ET)

Ellagic tannins were determined colorimetrically by the formation of a colored product in reaction with nitrous acid [21]. Solutions containing approximately 0.02% of ellagic tannins in 50% aq. MeOH (2.0 ml) and 0.16 mL of 6% HOAc were placed in a 1 cm cuvette, oxygen-free N_2 was bubbled for about 15 min. 0.16 ml of 6% aq. $NaNO_2$ was added, Nitrogen passed for 15 s and then the cell was sealed. The absorption was measured using Pedunculagin $C_{34}H_{24}O_{22}$ (1,6,2,3-hexahydroxydiphenoyl-D-glucose) as a standard. The reaction tubes were kept at room temperature (20 °C) for a further 24 h. The change in color from blue to the orange-yellow product was measured spectrophotometrically at 430 nm [22].

Gallotannins (GT)

In order to evaluate the content of gallotannins, the colorimetry method with Rhodanine (2-thio-4-ketothiazolidine, Sigma-Aldrich) was used [23]. The lyophilized murta seeds (150 mg) were extracted for 30 min using 10 ml of 70% (v/v) acetone/water in a sonicator at 4 °C. The extract was vacuum filtered through a coarse sintered glass filter into an ampule. The glass filter was washed with 5.0 ml of 2 N H_2SO_4, which was added to the ampule. The remaining solid was discarded. The sample was frozen in dry ice-isopropanol, the ampule was vacuum sealed and the sample was heated for 26 h at 100 °C. After hydrolysis, the ampule was opened and the entire sample was diluted to 50 ml with MiliQ water. The same hydrolysis treatment was used for commercial tannic acid (1,2,3,4,6-penta-*O*-{3,4-dihydroxy-5-[(3,4,5-trihydroxybenzoyl)oxy]benzoyl}-D-glucopyranose) (Sigma Aldrich), using 10 ml of 2 N H_2SO_4/mg of tannic acid in the hydrolysis and diluting the hydrolysate tenfold with water. In a graduated test tube, 1.5 ml of 0.667% methanolic rhodanine solution was added to 10 ml of sample. The sample consisted of l0 ml aliquot of the diluted hydrolysate or appropriate aliquots of the gallic acid (3,4,5-trihydroxybenzoic acid, Sigma Aldrich, Germany) standard made up to 1.0 ml with 0.2 N H_2SO_4. After exactly 5 min, 10 ml of 0.5 N aqueous KOH solution was added. After 2.5 min, the mixture was diluted to 25 ml with distilled water. Five to 10 min later, the absorbance at 520 nm was read.

Protein-precipitable phenolics (PPP)

The protein (bovine serum albumin, lyophilized powder, VWR, Germany) precipitating capacity, measured as protein-precipitable phenolics, was determined as previously described [21, 24]. Results were presented as tannic acid equivalents. To determine protein-precipitable phenolics, the extracts were concentrated ten times by removal of methanol *in vacuum* at 37 °C, followed by freeze-drying. The results were obtained from regression coefficients (slopes) of linear regression fitted to measurements performed to four different concentrations on three separate extracts, as suggested by other authors [25].

HPLC–MS/MS analysis

The chromatographic and mass spectrometric analysis was performed on a Shimadzu Prominence Mass Spectrometer Applied Biosystems/MDS Sciex 3200 Qtrap. The determination was made with an electrospray ionization source (ESI): Electrospray Turbo VTM (380 ºC) by isocratic elution with a mixture of methanol/water (70:30) plus formic acid 1% v/v (pH 4) as the mobile phase. A chromatography column RP-C18 Inertsil ODS-3 (2.1 × 150 mm, 3 mm) was used, the injection volume was 10 µl at a constant flow of 0.2 ml/min. The column temperature was 35 °C, the mobile phase was degassed by vacuum filtration through a 0.45 µm filter before use, and was used a 1.0 ml/min flow rate. The time for each analysis run was only 6.0 min. The injection volume was 20 µl. The eluent was split so that 30% was introduced into the inlet of the mass spectrometer and a divert valve was used to divert the eluent to waste from 0 to 3.2 min. Five minutes of balance was used between each sample measurement. The HPLC and spectrometer control were carried out using the Analyst 1.5.1 software. The corresponding condition is described as follow: Gas cur: 20 psi, CAD gas: Medium Gas1: 60 psi, Gas2: 30 psi

Ionization voltage: 4500 V. The analysis of the samples was carried out in both polarities using the EMS-EPI (Enhanced Mass Scan) method that corresponds to a general scanning scan covering a mass range from 100 to 1000 m/*z* which in turn has been associated with an EPI which will finally deliver a fragmentation trace of the target compounds. MS–MS conditions were optimized as follows: the source temperature at 120 °C, desolvation nitrogen gas temperature at 350 °C with a flow of 560 h, Ar collision gas at 0.35 Pa. The cone voltage used was 35 V, and the collision energy was optimized to be 22 eV. The multiple reaction monitoring (MRM) mode was used to quantify the analyses: the assay of investigated compounds was performed following two or three transitions per compound, the first one for quantitative purposes, and the second and third ones for confirmation.

Determination of the minimal inhibitory concentration

For this assay, three Gram positive (*S. aureus, B. cereus, and S. pyogenes)* and three Gram negative (*E. coli, S. typhi, P. aeruginosa*) bacterial strains, coming from Universidad de Santiago de Chile strain collection, were used. All of them were cultivated in MRH broth before use. Minimal inhibitory concentration (MIC) was determined by the microdilution broth method, as recently described [26]. Generally, 100 µl of suitable broth medium (MRS broth for LAB and MH broth for food-borne pathogenic bacteria) was added into a 96-well plate. Then, 100 µl of samples (10 mg/ml, different concentrations for antibiotics and phenolic compounds) were added and serially double-diluted. Finally, 100 µl of a bacterial solution (1 × 106 CFU/ml) was added into each well and mixed. The well that contained broth medium inoculated with bacteria was used as a positive control, whereas a well only with broth medium was used as the negative control. The 96-well plate was incubated for 24 h at 37 °C. The growth inhibition of bacteria in each well was obtained by turbidimetry, and turbidity values higher than control were accepted as positive well. The minimum inhibitory concentration (MIC) values are expressed as mg/ml.

Statistical analysis

The statistics program SPSS v. 5.0 was used for data analysis. All values are reported as means of five measurements, and their standard deviations (SD) are reported.

Results and discussion

Proximate analysis

The aim of this work is the evaluation of the chemical composition of murta seeds, to investigate the most relevant nutritional properties of this biowaste. Results from the proximal analysis are presented in Table 1. All parameters are expressed in fresh weight (FW) base, but in some cases, previously published data are reported on the dry weight (DW) base.

The proximate composition of murta seed was determined. It was found that seeds have a high dry matter content, which is similar to that reported for other berry seeds, such as black currant (93.5%) [17]. Such value was 1.5 times higher than reported for Amaranthus seed and 1.3 times higher than reported for cereal seeds [27]. Interestingly, murta seed contains a high level of crude protein, which is similar to those of more conventional grains, such as wheat, rice, corn, and barley (11%). This protein amount is 1.2 times lower than that of *Amaranthus* spp. [27]. but higher than other berry seeds [17]. The seed oil content is nearly 1.9 times higher than in *Amaranthus* spp. and 4.3 times higher than in cereals [27]. However, it is twice lower than that found in black currant berry seeds [17]. The ash content is very similar to that found in cereal seed (1.7%), but only about half as much of the ash content reported in *Amaranthus* species (2.9%) [27]. Moreover, this content is 2.3 times lower than that found in black currant berry seeds. Furthermore, murta seeds show small Vitamin C content, which is lower than found in other berry seeds, e.g., strawberry (5.7 times), rosehip (6.9 times), cranberry (18 times), and bilberry (26.2 times) [17], respectively. Also, glucose

Table 1 Chemical composition of murta seeds

Component	Value (g/100 g fresh weight)
Dry matter	93.8 ± 0.1
Raw fiber	64.0 ± 2.0
Oils	14.0 ± 1.0
Crude protein	12.0 ± 1.0
Other soluble sugars	2.3 ± 0.2
Vitamin C (mg/100 g FW)	30 ± 10
Energy value (cal/100 g FW)	427.0 ± 9.0
Ash	1.5 ± 0.1
Ca (mg/100 g FW)	42 ± 30
Mg (mg/100 g FW)	90 ± 40
Na (mg/100 g FW)	61 ± 30
K (mg/100 g FW)	180 ± 10
P (mg/100 g FW)	230 ± 10

(0.009–0.025%) and fructose (0.007–0.018%) have been reported as the main soluble sugars in murtilla seeds. In addition to the monosaccharides, the presence of sucrose disaccharide was also determined (data not shown). The same sugar combination was recently found in manguba seeds [28].

Mineral

Ashes were analyzed to quantify the minerals. The results are shown in Table 1. It can be observed that seeds are rich in several dietary essential minerals, such as calcium (0.042%), phosphorous (0.23%), and potassium (0.18%). Lower amounts of other minerals, such as magnesium and sodium, have been identified. The high mineral content demonstrates the potential for murta seeds to be used as a natural mineral source in the human diet [29].

According to the proximate composition, the energy value is 427 cal/g, which can be due to the high fiber content.

Fiber

Dietary fiber is classified into two categories according to its water solubility: insoluble dietary fiber (IDF) such as cellulose, parts of hemicellulose and lignin, and soluble dietary fiber (SDF) such as non-cellulosic polysaccharides, oligosaccharides, pectins, β-glucans and gums [30]. The SDF is widely recognized for its nutritional properties. Dietary fiber represents the most abundant constituent of murta seed, accounting for 68.2% of the dry matter (Table 1), which is lower than reported for other berries seeds like a rosehip seed (71.3%), but higher than in cranberry seeds (48.5%), as well as bilberry, strawberry and elderberry seeds that contain between 58.6 and 59.4%, respectively [17].

Four independent samples were analyzed to determine the composition of murta IDF (Table 2). The most abundant components of murta seed fiber are lignin and cellulose, and their total content reaches a value of 88.3% of the dietary fiber. It means that IDF was the most relevant constituent of murta seed fiber. On the other hand, SDF (pectin) represents only 11.3% of dietary fiber. The murta seed lignin content is nearly 1.5 times higher than previously reported in murta pulp [5] but similar to that found in jujube seeds and 1.3 times lower than in guava seeds [31]. The cellulose content in murta seed is smaller than in guava seed (28%) and jujube seed (37.3%). The hemicellulose content in guava (15.5%) and jujube seed (25.9%) are also higher than in murta seed [31]. Pectin content is slightly lower than reported in cacao pod husk (10.1%), which is also a byproduct of the cacao industry [32]. Nevertheless, it is four times lower than previously reported for murta fruit. Murta pectin is composed of homogalacturonan chains (DE 57 mol%; MW 597 kDa), which contains small rhamnogalacturonan insertions with galactose and arabinose side chains [5].

Table 2 Chemical composition of IDF and fatty acids of murta seeds

Component	Murta seed (g/100 g dry weight)
Lignin	32.1 ± 0.2
Cellulose	22.2 ± 0.3
Hemicellulose	2.2 ± 0.3
Pectin	7.5 ± 0.1
Fatty acid (common name)	
C16:0 palmitic acid	2.5 ± 0.3
C18:0 stearic acid	0.8 ± 0.1
C18:1 oleic acid	7.7 ± 0.2
C18:2 linoleic acid	88.2 ± 0.9
C18:3 alpha-linolenic acid	0.8 ± 0.1
Total saturated fatty acids (SAFAs)	3.3 ± 0.4
Total monounsaturated fatty acids (MUFAs)	7.7 ± 0.2
Total polyunsaturated fatty acids (PUFAs)	89.0 ± 0.9

According to these results, murta seeds could be considered as a dietary fiber supplement for the human diet. It is known that dietary fiber intake has a physiological impact on the prevention and management of human obesity and related chronic diseases, such as Metabolic Syndrome (abdominal obesity, high blood pressure, hyperglycemia/insulin resistance, and dyslipidemia), constipation and the increased risk of coronary heart disease and prostate cancer [33]. Furthermore, the consumption of dietary fiber (20–30 g per day) is associated with health benefits.

Fatty acids composition

Table 2 shows the fatty acids composition of this seed. Fatty acids are essential constituents of murta seeds, accounting for 14% of the fresh weight. This content is higher than reported for grape seeds (4.5–11.1%) [34] and some vegetable oils [35]. Murta seeds are rich in many essential oils, mainly the unsaturated linoleic (88.2%) and oleic acids (7.7%). Their healthy properties are recognized for the prevention of cardiovascular diseases [36, 37]. Murta seeds constitute a valuable source of the oils mentioned above.

Protein and amino acids composition

The murta crude protein content is higher than guava seeds (7.6%) but lower than in tomato seeds (17.6%), or watermelon seeds (25.2–37.0%) [38, 39]. Figure 1 shows the amino acid composition results and in Table 3, the protein quality parameters of murta seed are shown.

The most frequent amino acids in murta seed are glutamine plus glutamic acid (16.6%), arginine (15.9%), asparagines and aspartic acid (8.9%) (Fig. 1). The first two amino acids are coincident in abundance with those of guava seed protein. Murta seed shows the highest essential amino acid content (EAA) (33.4%) among studied seed proteins (Table 3). The EAA value is close to those reported for faba bean and quinoa seed, but 1.4 times higher than guava seed. Histidine content, which is essential for childhood nutrition, human growth, and tissue repair, is about two times more elevated than daily intake suggested by FAO/WHO/UNU [16]. The tryptophan content (7.1%) in murta seed protein is 12 times higher than daily reference intakes for adults and 3.7 times higher than guava protein.

Except for methionine, lysine, and threonine, the essential amino acid profile for murta seed protein is 1.4 times higher than the intake recommended by FAO/WHO study for adults. The PER of murta seed is the highest found in compared proteins. Furthermore, the sulfur-containing amino acids cysteine and methionine are the sequence limiting amino acids in all proteins. It is interesting to note that both murta and guava proteins show lysine as the third sequence

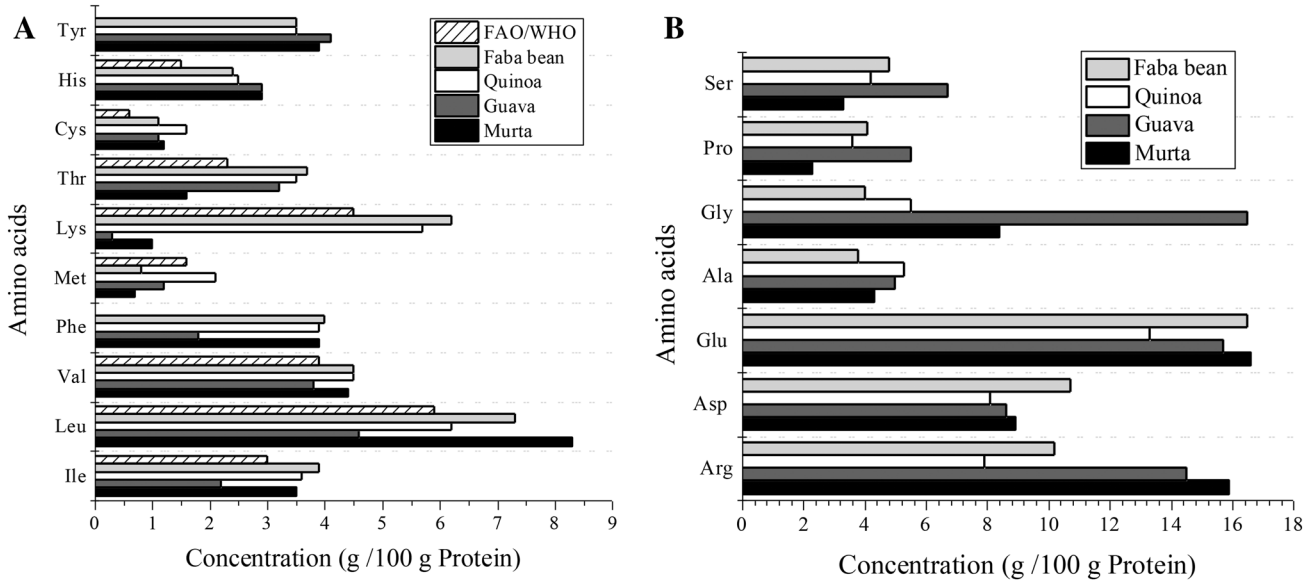

Fig. 1 Comparison of FAO/WHO amino acid composition suggested for human consumption for murta and referential seeds

Table 3 Amino acid quality of murta seed proteins compared with referential seeds

Amino acid	Murta	Guava	Quinoa	Faba bean	FAO/WHO (2011)[a]
SAA	1.9	2.3	3.7	1.9	2.2
AAA	34.7	25.5	23.5	26.3	6.6
EAA	33.4	21.9	32	32.8	23.3
NEAA	64.8	77.7	53	58.7	–
Protein nutritional quality					
PER	2.9	1.2	2.0	2.5	2.2
EAAI	93.2	91.2	81.8	73.2	100
NI	17.0	17.3	10.6	26.0	–
BQ[a]	86.6	84.3	74.0	64.6	94.0
CS (%)	43.8	6.7	100	50.0	43.8
Sequence limiting	I Met	I Lys	I Cys	I Met	I Cys+Trp
Amino acid	II Lys	II Cys	II Met	II Cys	II His
	III Cys	III Met	III His	III His	III Met

SAA sulphur amino acids, *AAA* aromatic amino acids, *EAA* essential amino acids, *NEAA* Non-essential amino acids, *PER* protein efficiency ratio, *EAAI* essential amino acid-index, *NI* nutritional index, *BQ* biological quality expressed as the ratio of essential amino acids to whole egg amino acids, *CS* protein chemical score

[a]Values suggested for adult compsumption

limiting essential amino acid, which could be related to these plants belong to the same *Myrtaceae* family.

On the other hand, quinoa seed and faba bean show the same sequence limiting amino acids as FAO/WHO protein, but with a different order. Histidine is the third common essential amino acid, but tryptophan also appears as a limiting amino acid in FAO/WHO protein. The NI of murta seed is lower than in faba bean, very close to guava seed but higher than that of quinoa. Finally, BQ of murta seed is the highest for all compared proteins. These facts suggest a potential use of murta seeds as an alternative source of essential amino acids and high-quality proteins for human nutrition [29].

Polyphenols, tannins and antioxidant properties

During the last decades, research on plant polyphenols has received considerable interest due to its safer ingredients for food preservation and added beneficial effects on human health [40, 41]. Results of total polyphenolic content (TPC) and tannin fraction in murta seed extracts, as well as their antioxidant capacity, are reported in Table 4.

The Folin–Ciocalteu method has been the most common assay used to estimate TPC in many plants, fruits, and foods due to its simplicity and reproducibility. The results show that murta seeds are rich in polyphenolic compounds (234 ± 19 mg GAE/100 g FW). This value is close to those found in blackberry seeds (170–305 mg GAE/100 g FW) [42] and higher than those found in several berry seed press residues (90–221 mg GAE/100 g FW) [17] and grape seeds (7–161 mg GAE/100 g FW) [43]. In contrast, murta seed TPC is lower than recently found in other berries grown in Chile such as blueberries (480–850 mg GAE/100 g FW), strawberries (630–850 mg GAE/100 g FW) and maqui berry (1230–1580 mg GAE/100 g FW) [44]. The results of polyphenol content obtained in present work for murta seed confirms that both murta fruit and seed are good sources of bioactive compounds. Other authors [1, 45] reported TPC values in murta fruit and leaves ranging from 1900 to 5600 mg GAE/100 g FW. These values are also higher than obtained for murta seed of present work, suggesting that polyphenolic content in different organs of murtilla plants follows this order: fruit > leaves > seeds.

On the other side, the murta seeds comprise a significant fraction (1.2 mg/100 g FW) of condensed tannins (proanthocyanidins). Hydrolyzable tannin fraction is constituted by ellagitannins (0.8 mg/100 g FW) and gallotannins (0.2 mg/100 g FW). Both groups of polyphenols have been detected in several tropical and temperate seeds [46] and they are reported as a biomarker of *Myrtaceae* [47]. In general, tannin fractions are associated with the nutritional/antinutritional quality of many fruits, pods, roots, flowers, seeds, and tubers [48]. In general, condensed tannin content between 0.1 and 2.0 mg/100 g DW induces a positive response in animals and humans, and in murta seed, this value is within this range. In contrast, tannin values between 2 and 12 mg/100 g DW are often associated with gastrointestinal disorders, enzyme inactivation and decreased intake tested in several animal models [49, 50]. The beneficial or detrimental effect of tannins depends on several factors, such as the capacity to inactivate proteins, the molecular weight, the monomer-type, and the features of the digestive systems [51]. Besides, hydrolyzable tannins are recognized as antinutritional factors in a much lower concentration, in comparison to condensed tannins [52]. That is why complimentary research should be done to establish the potential toxicological/antinutritional risk of murta seed tannin fraction.

Moreover, the antioxidant activity of murta seed determined by the ORAC method shows an excellent antioxidant capacity (4012 µmol TE/100 g). It has been reported for Chilean murta fruit an ORAC value ranging from 9698 to 12,456 µmol TE/100 g, which is higher than obtained for different blueberry fresh fruit [53].

HPLC–MS/MS analysis of seed extract

Polyphenols are a group of natural compounds widely distributed in plants. Regardless of the high distribution in the plant kingdom, little research has been done to characterize agroindustrial polyphenol-rich biomass from the nutritional perspective [54, 55].

The polyphenolic profile of *U. molinae* leaves and fruits assessed by several spectroscopic and chromatography

Table 4 Polyphenolics content and tannin fractions of murta seeds

Sample	TPC[a] (mg/100 g FW)	Condensed tannins[a] (mg/100 g FW)	Ellagitannins (mg/100 g FW)	Gallotannins (mg/100 g FW)	Protein precipitable phenolics (mg/100 g FW)
Murta seed	234.0 ± 19.0	1.2 ± 0.3	0.8 ± 0.4	0.2 ± 0.1	2.9 ± 0.5

TPC determined for standarization of antimicrobial activity purpose

[a]Condensed tannins expressed as proanthocyanidins

techniques are well-known [56]. However, to our best knowledge, the information regarding the qualitative analysis regarding the murta seeds polyphenolic composition is scarce. Table 5 shows the chemical composition of each compound detected by HPLC–MS/MS, and Fig. 2 shown the chemical structure of identified components in murta seed extract.

The obtained results show that polyphenolic profiles in murta seeds are different in comparison to polyphenol biomarkers in leaves, fruits, and biomass of murta reported in the literature [57]. The identification of each peak was possible via retention time as well as MS spectra of samples and comparison with authentic standards.

The structural features of various detected phenolics compounds contained in murta seeds should contribute to the potential biological activity of the extract. It is worth noticing that HPLC–MS/MS experiments confirm a significant level of phenolics, proanthocyanidins, and tannins, previously quantified by colorimetric determinations.

So far, there is no previous information regarding low- and high-molecular-weight phenolic from *U. molinae* seeds in the literature. However, other studies reported phenolic compounds profile in seeds from other related *Myrtaceae* species and also in murta ecotypes fruits [58]. Considering the main findings, phenolic acids, anthocyanidins, and flavonoids glycosides seem to be quite common in murta ecotypes [59–61]. Moreover, it would be expected to found hydrolyzable tannins in murta seed extracts analysis [56]; however, oligomeric polyflavonoids might be undetectable, depending on the analysis conditions. That is why another analytic condition may be necessary to detect a broader spectrum of polyphenolic compounds from murta seeds.

Antimicrobial activity

The chemical composition of murta seed extract demonstrates the presence of secondary metabolites (phenolic acids, flavonoids, and tannins) that are often produced by plants as a defense strategy against pathogens attack. There are several reviews about the fungicidal and bactericidal activity of plant extracts rich in such phytochemicals and their potential application as natural antimicrobial products [62, 63]. However, to the best of our knowledge, there are no reports on the antibacterial activity of murta seed extracts. For this reason, the effect of murta seed extract on bacterial strains related to human health, such as Gram positive bacterias: *S. aureus, B. cereus, and S. pyogenes* were studied. Those strains are highly pathogenic for humans and sometimes are associated with chronic dermal wounds [64]. Likewise, three strains of Gram negative bacterias: *E. coli, S. typhi, P. aeruginosa*, described as food contaminants, were also subjected to antimicrobial activity tests. The minimum inhibitory concentration (MIC) of the hydroalcoholic extract

Table 5 Chemical composition of each compound identified by HPLC–MS in murta seed extract

Compound	$[M–H]^-$ (m/z)	MS^2 (m/z)	Concentration (mg/100 g DW)
Gallic acid	168	124, 79	63.80 ± 2.10
Syringic acid	197	182, 123	3.65 ± 0.51
Ferulic acid	193	147, 103	2.79 ± 0.31
Chlorogenic acid	353	191, 85	0.84 ± 0.02
Caffeic acid	179	135, 134	1.08 ± 0.09
Catechin	289	245, 109	2.31 ± 0.01
Quercetin	301	151, 179	6.70 ± 0.12
Luteolin	285	133, 151	0.09 ± 0.01
Vanillic acid	167	123, 152	48.20 ± 0.90
Epicatechin	289	243, 109	1.67 ± 0.02
Abscisic acid	263	153, 219	0.28 ± 0.01
Pedunculagin	783	481, 301, 275, 249, 229	NQ
Castalagin/vescalagin	933	631, 451, 301	NQ
Quercetin-3-*O*-rhamnosyl-rhamnosyl-galactoside	755	273, 291, 301, 363, 435, 453	NQ
Quercetin 3-*O*-β-D-(6″-galloyl) galactopyranoside	615	463, 301	NQ
Jaceosidin (4′.5.7-Trihydroxy-3′.6-dimethoxyflavone)	329	314, 299, 269, 146	NQ
5.6.7.4′-tetramethoxyflavone	341	326, 311, 283	NQ
Quercetin-3-*O*-glucoside-7-*O*-rhamnoside	609	463, 301	NQ

NQ = identified but not quantified

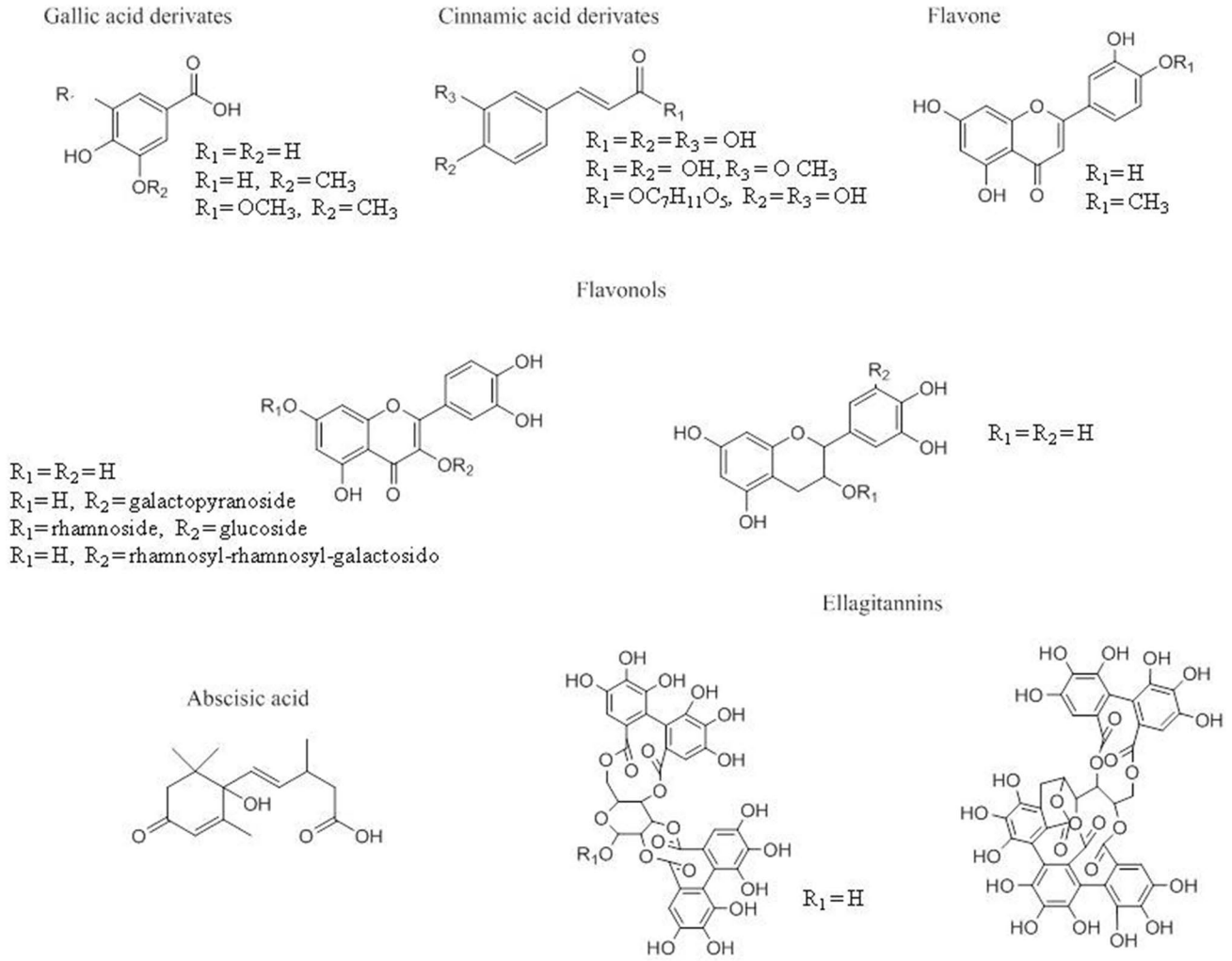

Fig. 2 Chemical structure of the compounds identified in murta seed extract

of murta seeds was in a concentration range between 0.1 and 12 mg GAE/ml and the results are presented in Table 6.

It could be seen that MIC is below 1 only for *S. aureus* (G positive) and *P. aeruginosa* (G negative) strains (0.4–0.6 mg GAE/ml), indicating higher susceptibility of those bacterial strains to murta seed extract phytochemicals. On the other side, MIC values above 1 mg GAE/ml are obtained for all other tested bacterial strains, including both Gram+ and Gram-. It is known that most commercial antibiotics are active at concentration range from 0.008 to 0.025 mg/ml [65]. Murta seed extract is found to be effective in the inhibition of *S. aureus* (G positive) and *P. aeruginosa* (G negative) strains and could be useful to control them.

In preliminary work, the antimicrobial activity of murta leaves extracts against *P. aeruginosa, S. aureus, E. aerogenes,* and *C. albicans* were studied [66]. Standardized extracts were analyzed at concentrations of about 527 mg GAE/ml, which is 44 times higher than tested in this work, explaining the higher inhibitory activity obtained in all bacterial strains evaluated. This extract was used as a natural

Table 6 Minimum inhibitory concentration of murta seed extract on Gram+ and Gram− bacterial strains

Sample	MIC (mg GAE/ml)			MIC (mg GAE/ml)		
	Gram+			Gram−		
	S. aureus	*B. cereus*	*S. pyogenes*	*E. coli*	*S. typhi*	*P. aeruginosa*
Murta seed extract	0.4 ± 0.1	> 1.2	> 1.2	1.2 ± 0.1	1.2 ± 0.1	0.6 ± 0.1

ingredient in cosmetic creams preparation, and it was able to replace paraben and other synthetic antioxidants as a preservative agent. In another work, the antibacterial effect of alcoholic extracts of murta leaves was assessed and their potential use as natural preservatives had been proposed [67]. On the other hand, the antibacterial effect of murta fruit extracts against both *E. coli* and *S. typhi* strains has been reported [68]. They found that a murta fruit extract had an inhibitory effect similar to several commercial synthetic antibiotics and also proposed their use as a food biopreservative.

Moreover, the activity of single polyphenolic compounds against *P. aeruginosa* strain has been reported, finding MIC values higher than for the hydroalcoholic extract of murta seeds [69]. The latter could indicate the existence of synergism between the compounds identified in seed extract. Then, the highest antibacterial activity of seed extracts against the nosocomial pathogens encourages their use as potential antibacterial agents for wound healing.

Differences observed in the bactericidal activity of murta extracts from different plant parts (seeds, leaves, fruits) could be related to the extract chemical composition, concentrations of bioactive components in the extracts and extract polyphenolic concentrations employed in the antimicrobial test. For example, methanolic extracts of stems and leaves of the Myrtaceae plant *Amomyrtus Meli* (used in traditional medicine Huilliche), showed no activity against *S. aureus*, *E. coli,* and *P. aeruginosa* [65]. In general, these results indicate that *Ugni molinae* is a plant rich in secondary metabolites with antibacterial activity and that these compounds have a wide distribution within the murta plant, in leaves, fruits, and seeds. Finally, murta extracts could found potential uses in the cosmetic, pharmaceutical, and food industries.

Conclusions

The present study demonstrated the presence of valuable nutritional compounds in murta seeds. The seed is rich in dietary fiber, minerals, and proteins with high nutritional quality. The concentration of essential amino acids fulfilled the requirements for human consumption. The oil fraction was mainly composed of polyunsaturated fatty acids. For the first time, it is reported polyphenolic profile in murta seeds and results provided excellent antioxidant capacity. Besides, there is a growing interest in identifying new natural, low-cost antioxidants for commercial use to improve human health. According to the chemical composition, the murta seed is also a valuable source of fiber and polyphenolic compounds, which are both highly desirable for the formulation of functional foods. Moreover, high bactericidal activity toward selected Gram negative and Gram positive pathogens increase the applicative potential of these seeds and its extracts to not only the food industry but also biomedical and cosmetic application.

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Compliance with ethical standards

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

References

1. T. Augusto, F. Pirce, E. Scheuermann, M. Spoto, T. Vieira, Grasas Aceites **68**(1), 83 (2017)
2. C. Fredes, *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromática* (Universidad de Santiago de Chile, Santiago, 2009), p. 469
3. E. Scheuermann, I. Seguel, A. Montenegro, R. Bustos, E. Hormazábal, A. Quiroz, J. Sci. Food Agric. **88**(3), 485 (2007)
4. I. Seguel, E. Peñaloza, N. Gaete, A. Montenegro, A. Torres, Agro Surf. **28**, 32 (2000)
5. E. Taboada, P. Fisher, R. Jara, E. Zúñiga, A. Gutierrez, J.C. Cabrera, M. Gidekel, R. Villalonga, G. Cabrera, Food Chem. **123**, 669 (2010)
6. B. Waltenberger, M. Halabalaki, S. Schwaiger, N. Adamopoulos, N. Allouche, B.L. Fiebich, N. Hermans, P. Jansen-Dürr, V. Kesternich, L. Pieters, S. Schönbichler, A.-L. Skaltsounis, H. Tran, J.P. Trougakos, A. Viljoen, J.-L. Wolfender, C. Wolfrum, N. Xynos, H. Stuppner, Molecules **23**(5), 1097 (2018)
7. S.H. Nile, S.W. Park, Nutrition **30**(2), 134 (2014)
8. P. Cuniff, *In official methods of analysis*, 16th edn. (Association of Official Analytical Chemistry International, Virginia, 2005)
9. W. Horwitz, G.W. Latimer, *In official methods of analysis of AOAC International*, 18th edn. (Association of Official Analytical Chemistry International, Maryland, USA, 2010)
10. M.K. Dubois, A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Anal. Chem. **28**, 350 (1956)
11. E.V. Crisan, A. Sands, *Biology and Cultivation of Edible Mushrooms* (Academic Press, New York, 1978), p. 137
12. P.J. Van Soest, J.B. Robertson, B.A. Lewis, J. Dairy Sci. **74**(10), 3583 (1991)
13. N. Blumenkrantz, G. Asboe-Hansen, Anal. Biochem. **54**, 484 (1973)
14. J. Landry, S. Delhaye, D.G. Jones, J. Sci. Food Agric. **58**, 439 (1992)
15. R.H. Alsmeyer, A.E. Cunningham, M.L. Happich, Food Technol. **28**, 34 (1974)
16. FAO/WHO/UNU, *Protein and amino acid requirements in human nutrition. Technical report series number 935* (WHO Press, Geneva, 2011)
17. D. Helbig, V. Böhmb, A. Wagner, R. Schubert, G. Jahreis, Food Chem. **111**, 1043 (2008)
18. K. Schlesier, M. Harwat, V. Böhm, R. Bitsch, Free Radic. Res. **36**(2), 177 (2002)
19. S. Dudonné, X. Vitrac, P. Coutière, M. Woillez, J. Merillon, J. Agric. Food Chem. **57**, 1768 (2009)
20. R.L. Prior, H. Hoang, L. Gu, X. Wu, M. Bacchiocca, L. Howard, M. Hampsich-Woodill, D. Huang, B. Ou, R. Jacob, J. Agric. Food Chem. **51**, 3273 (2003)

21. P.E. Shay, J.A. Trofymow, C. PeterConstabel, Plant Methods **13**, 63 (2017)
22. E.C. Bate-Smith, Phytochemistry **11**, 1153 (1972)
23. K.H. Inoue, A.E. Hagerman, Anal. Biochem. **169**(2), 363 (1988)
24. A.E. Hagerman, L.G. Butler, J. Agric. Food Chem. **26**(4), 809 (1978)
25. J.S. Martin, M.M. Martin, Oecologia **54**(2), 205 (1982)
26. C.L. Chana, R.Y. Gana, N.P. Shah, H. Corke, Food Control **92**, 437 (2018)
27. M. Marcone, F. Jahaniaval, H. Aliee, Y. Kakuda, Food Chem. **81**, 7 (2003)
28. A. Pereira, G. Araujo, P. Henrique, H. Silvano, M. Nogueira, G.M. Pastore, Food Res. Int. **121**, 880 (2019)
29. M. Edelman, M. Colt, Front. Chem. (2016). https://doi.org/10.3389/fchem.2016.00032
30. Y.O. Li, A.R. Komarek, Food Qual. Saf. **1**(1), 47 (2017)
31. A. Ouensanga, L. Largitte, M. Arsene, Microporus Mesoporus Mater. **59**, 85 (2003)
32. L. Vriesmann, R. Teofilo, C. Oliveira, LWT Food Sci. Technol. **49**, 108 (2012)
33. J.-P. Chen, G.-C. Chen, X.-P. Wang, L. Qin, Y. Bai, Nutrients **10**(1), 24 (2018)
34. F. Al Juhaimi, Ü. Geçgel, M. Gülcü, M. Hamurcu, M.M. Özcan, S. Afr. J. Enol. Viticult. **38**(1), 103 (2017)
35. J. Orsavova, L. Misurcova, J.V. Ambrozova, R. Vicha, J. Mlcek, Int. J. Mol. Sci. **16**(6), 12871 (2015)
36. R. Jandacek, Healthcare **5**(2), 25 (2017)
37. T. Ederich, M. Ramos-da Silva, A. Camacho, A. Aline Marcadenti, A. Machado, J. Int. Soc. Sports Nutr. **12**(1), 36 (2015)
38. R. Noor, J. Marikkar, I. Amin, M. Shuhaimi, Int. J. Food Prop. **18**(11), 2380 (2015)
39. V. Nour, T.D. Panaite, M. Ropota, R. Turcu, I. Trandafir, A.R. Corbu, CyTA J. Food **16**(1), 222 (2018)
40. J. Nowshehri, A. Bhat, M. Shah, Food Res. Int. **77**(3), 333 (2015)
41. M. Carocho, P. Morales, I. Ferreira, Trends Food Sci. Technol. **71**, 107 (2017)
42. I. Koca, B. Karadeniz, Sci. Hortic. **121**, 477 (2009)
43. M. Anastasiadi, H. Pratsinis, D. Kletsas, A.L. Skaltsounis, S.A. Haroutounian, Food Res. Int. **43**, 805 (2010)
44. C. Fredes, G. Montenegro, J.P. Zoffoli, F. Santander, P. Robert, Cienc. Investig. Agrar. **41**(1), 49 (2014)
45. C. López, F. Bustos, X. Valenzuela, G. López-Carballo, J. Vilariño, M.J. Galotto, Food Res. Int. **102**, 119 (2017)
46. A.M. Díaz, G.V. Caldas, M.W. Blair, Food Res. Int. **43**(2), 595 (2010)
47. F. Nasrin, R. Pandian, Int. J. Adv. Sci. Eng. Technol. **4**, 132 (2015)
48. L. Geny, C. Saucier, S. Bracco, F. Daviaud, Y. Glories, J. Agric. Food Chem. **51**(27), 8051 (2003)
49. A. Ishisaka, S. Ichikawa, H. Sakakibara, Free Radic. Biol. Med. **51**, 1329 (2011)
50. K. Selvakumar, R.L. Prabha, K. Saranya, S. Bavithra, G. Krishnamoorthy, J. Arunakaran, Hum. Exp. Toxicol. **32**, 706 (2013)
51. N.M. Delimont, M.D. Haub, B.L. Lindshield, Curr. Dev. Nutr. **182**, 1 (2017)
52. P. Frutos, G. Hervás, F.J. Giráldez, A.R. Mantecón, Span. J. Agric. Res. **2**(2), 191 (2004). https://doi.org/10.5424/sjar/2004022-73
53. INTA. Public database of polyphenolic content and antioxidant capacity (ORAC) of fruits. http://www.portalantioxidantes.com/orac-base-de-datos-actividad-antioxidante-y-contenido-de-polifenoles-totales-en-frutas (2015).
54. J. Figueroa, J. Borrás-Linares, A. Lozano-Sánchez, Food Res. Int. **105**, 752 (2018)
55. B. Singh, J. Singh, A. Kaur, N. Singh, Food Res. Int. **101**, 1 (2017)
56. I. Quispe-Fuentes, A. Vega-Gálvez, M. Aranda, J. Sci. Food Agric. **98**(11), 4168 (2018)
57. M. Avello, E. Pastene, M. González, M. Bittner, J. Becerra, Rev. Cuba. Plant. Med. **18**(4), 596 (2013)
58. M. Plaza, Â.G. Batista, C.B. Cazarin, M. Sandahl, C. Turner, E. Östman, M.R. Maróstica, Food Chem. **211**, 185 (2016)
59. M. Rubilar, M. Pinelo, M. Ihl, E. Scheuermann, J. Sineiro, M.J. Nuñez, J. Agric. Food Chem. **54**(1), 59 (2006)
60. S. Alfaro, A. Mutis, R. Palma, A. Quiroz, I. Seguel, E. Scheuermann, J. Soil Sci. Plant. Nut. **13**(1), 67 (2013)
61. M. Chacón-Fuentes, L. Parra, C. Rodriguez Saona, I. Seguel, R. Ceballos, A. Quiroz, Environ. Entomol. **44**(3), 627 (2015)
62. F.J. Álvarez-Martínez, E. Barrajón-Catalán, J.A. Encinar, J.C. Rodríguez-Díaz, V. Micol, Curr. Med. Chem. (2018). https://doi.org/10.2174/0929867325666181008115650
63. M. Daglia, Curr. Opin. Biotechnol. **23**(2), 174 (2012)
64. M.S. Hu, M.R. Borrelli, H.P. Lorenz, M.T. Longaker, D.C. Wan, Stem Cells Int. **690**, 2018 (1983). https://doi.org/10.1155/2018/6901983
65. P. Mølgaard, J.G. Holler, B. Asar, I. Liberna, L.B. Rosenbæk, C.P. Jebjerg, L. Jørgensen, J. Lauritzen, A. Guzman, A. Adsersen, H.T. Simonsen, J. Ethnopharmacol **138**, 219 (2011)
66. M. Avello, R. Valdivia, M.A. Mondaca, J.L. Ordoñez, M. Bittner, J. Becerra, Bol. Latinoam. Caribe Plant. Med. Aromát. **8**(2), 141 (2009)
67. C. Shene, A. Reyes, M. Villarroel, J. Sineiro, M. Pinelo, M. Rubilar, Eur. Food Res. Technol. **228**(3), 467 (2009)
68. M.P. Junqueira-Gonçalves, L. Yáñez, C. Morales, M. Navarro, R.A. Contreras, G.E. Zúñiga, Molecules **20**, 5698 (2015)
69. P. Jayaraman, M.K. Sakharkar, C.S. Lim, T.H. Tang, K.R. Sakharkar, Int. J. Biol. Sci. **6**(6), 556 (2010)