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ORIGINAL PAPER

Plant location and extraction procedure strongly alter the antimicrobial activity of murta extracts

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Abstract Leaves and fruits of Murta (*Ugni Molinae* Turcz.) growing in three locations of Chile with diverse climatic conditions were extracted by using ethanol/water mixtures at different ratios and the antimicrobial activity was assessed. Extracts containing the highest polyphenolic content were from murta plants grown nearer to the mountain (58 mg GAE/g murta), subjected to extreme summer/winter-day/night temperature changes and rainy regime. Extracts from leaves collected in the valley and coast contained 46 and 40 mg GAE/g murta, respectively. A mixture of 50% ethanol/water was the most efficient in extracting polyphenols, showing pure solvents—both water and ethanol—a lower extraction capacity. No correlation between antioxidant capacity and polyphenolic content was found. Extracts from Murta leaves provoked a decrease in the growing of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, and showed no activity against the beneficial, probiotic bacteria. A significant correlation between polyphenol content and antimicrobial activity on harmful bacteria was found. Myricetin glucoside and quercetin glucoside/glucuronide/dirhamnoside presumably contributed to the antimicrobial activity of the extract. The higher antimicrobial activity of leaves extracts compared to the fruits could be attributed to flavan-3-ols and other flavonol glycosides. Quercetin glucuronide, myricetin xyloside and flavan-3-ols in polymeric form were tentatively identified for the first time in murta extracts. Both extracts showed an antimicrobial activity similar to some commercial antibiotics, suggesting their suitability to replace synthetic antimicrobials in food.

Keywords Murta (*Ugni molinae* Turcz.) · Polyphenols · Antimicrobial activity · Food supplements · HPLC–DAD–MS · Flavonol glycosides

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Introduction

Murta is a wild shrub growing in the coast and pre-Andean mountains of South Chile. Mapuche native inhabitants have transmitted the health-beneficial properties of consumption of leaves for centuries. Murta fruits are also highly appreciated due to the pleasant flavor and aroma. The infusions have been traditionally used to lessen urinary tract pain, and as astringents, stimulants and phytoestrogenics [1]. Until recently, no scientific papers about the composition and health effects of murta extracts had been published. In 2006, two scientific papers reported the polyphenolic composition and the antiinflammatory properties of murta extracts, respectively [2, 3]. The interest for potential applications of murta compounds had risen considerably from then; at least ten different papers in peer reviewed journals have focused on murta components in the last 2 years. Among others, the analgesic and the protective effect against oxidative damage of human enterocytes have been reported [4, 5].

The potential uses of murta extracts as antimicrobial agents have not been reported yet. The use of natural antimicrobials for food applications is supported by the demanding need of replacing synthetic food additives. Food authorities are paying increasing attention to the regulation and uses of the so-called antimicrobial food additives. US Food and Drug Administration, for instance, requires chemical and biological identification of the antimicrobial agents and the group of target microbes for approval of the antimicrobial food additive [6]. In most of the cases, the beneficial health properties of murta extracts have been attributed to the presence of polyphenols [3].

Secondary metabolites such as tannins, flavonoids, alkaloids and several other aromatic compounds act as plant defense mechanisms against predation by many microorganisms. Some phenolic compounds have been reported to have antimicrobial activity against a wide spectrum of microorganisms. The antimicrobial activity of vegetal extracts containing flavonols, mainly rutin, has been reported by Fattouch et al. [7] in Tunisian *Cydonia oblonga* extracts, or by Pepeljnjak et al. [8] in *Pelargonium rodula* extracts. Also, flavanones have been found to be the most active antimicrobial agents in propolis [9].

Polyphenolic composition of plant extracts can vary dramatically depending on two main factors; the cultivation conditions and the solvent used for extraction [10]. Murta shrubs can grow in diverse climatic conditions, either under mild temperatures or subjected to extreme thermal amplitudes. It is known that plants which undergo high stress levels during cultivation generate a major range of phenolics as defensive mechanism [10]. Araucanía region in Chile is a small region in the South part of the country, which combines extreme temperature changes next to the pre-Andian mountain chain to mild climatic conditions nearer the coast. This makes Araucanía a suitable region to study the influence of climatic conditions on the phenolic profile of vegetal matrixes. It has been reported the different chemical properties (e.g. polarity, hydrophobicity) of polyphenols contained in murta [3]. Depending on the solvent used—methanol, ethanol or water—it is expected that the phenol profile of the extracts change substantially [11, 12].

In this work, both the antimicrobial (antibacterial and antifungal) and the antioxidant capacity of extracts from murta leaves and fruits were evaluated. Antimicrobial activity was evaluated on Gram positive and Gram negative bacteria and two fungi strains. The plant material has been collected in three different areas of the Araucanía region with diverse climatic conditions. Extraction has been performed with various ethanol–water ratios to evaluate the capacity of extraction of solvents with different polarity. HPLC–DAD–MS tentative identification of the phenolic compounds has been used to relate phenolic composition to antimicrobial activity.

Materials and methods

Extracts

Plant material (leaves and fruits) was collected during January 2007 from three different locations of the Araucanía region (Chile), which are close in distance but dramatically differ in climatic regime (see Table 1). The collected material was then dried in a stove at 35 °C to constant weight. Murta leaves and fruits were ground in a coffee grinder and sieved (Retsch, Germany). Particles with particle size ranged between 250 and 500 μm were used for the extraction. Sample (5 g) was macerated with the solvent (25 mL) by using a mortar and pestle at ambient temperature. A nitrogen flow was kept during the extraction in a hermetic cabinet to avoid polyphenol oxidation. After 24 h the extract was sieved with sterile cheesecloth and filtered through Whatman No. 1 filter paper. Filtrate was concentrated in a rotary evaporator at 35 °C (Heidolph, Laborota 4000, Germany), lyophilized to dryness for 24 h (DWS, Heto Holten A/S, Denmark), weighed for the determination of the soluble solids and stored at −18 °C until use. For antimicrobial activity tests dry powder was reconstituted in the solvent used for the extraction. Water to ethanol (v/v) in volumetric ratios of 100/0, 75/25, 50/50, 25/75 and 0/100 were used as extraction solvents. The solvents used for extraction, Milli-Q water and 99% pure ethanol were supplied by Sigma-Aldrich (Germany).

Table 1 Altitude and climatic features of the three locations from which murta samples were taken

Location	Name	Latitude	Longitude	Elevation (m asl)	Thermal amplitude	Rain regime
Andean mountain chain	Villarrica	39° 17′	72° 13′	232	Wide. Extreme temperature changes summer/winter-day/night	Highly rainy
Valley	Pitrufquén	38° 56′	72° 38′	98	Medium. Medium temperature changes summer/winter	Medium
Coast	Queule	39° 23′	73° 14′	6	Short. Little variations	Moderate

asl Above sea level

Microorganisms

Antimicrobial activity of murta extracts was evaluated on four bacteria; *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and two fungi; *Aspergillus niger* ATCC 20447 and a native *Penicillium expansum* strain, all of them considered harmful and undesirable in food. Murta extracts were also evaluated on three beneficial, probiotic bacteria; *Bifidobacterium longum* ATCC 15707, *Lactobacillus acidophilus* LA5, and *Streptococcus thermophilus* Th4. All microorganisms were supplied by Abiasa (Tui, Spain).

Antimicrobial activity

Bacteria were grown at 37 °C. Culture media for *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 was Tryptic Soy Broth (Merck, Darmstadt, Germany). Lactic acid bacteria were grown in MRS broth (Difco™, Becton, Dickinson and Co., Sparks, MD, USA) under anaerobic conditions (95% H_2 and 5% CO_2, GasPak Plus, BBL, Sparks, MD, USA). Tested bacteria were incubated for 24 h. Fungi were grown at 30 °C in Potato dextrose broth (Merck, Darmstadt, Germany) for 72 h. Cells (100 µL, 10^6 cells/mL) were inoculated onto the surface of the respective agar media. Dry filter paper disks (6 mm in diameter) saturated with the sterile tested extract (90 µL) were placed on the surface of each inoculated plate. Plates were incubated for different periods depending on the growth rate of the strain. Antimicrobial activity was determined by measurement of inhibition area around each paper disk. The control for each assay was the extraction solvent; these showed no growth inhibition. The inhibition zones produced by the plant extracts were compared to those produced by commercial standard antibiotics: Gentamicine (Oxoid, 10 µg) and Penicillin G (Oxoid, 10 IU). Gentamicine is used for treating many infections, especially those arising from the gastrointestinal and urinary tract or hospital pneumonias. Benzylpenicillin, also known as Penicillin G, is efficient against gonorrhea, meningitis, syphilis and septicemia.

Effect of the extracts on growth in liquid media

Two mL of sterilized culture media were supplemented with 100 µL of extract (sterilized by 0.2 µm filtration). Each tube was inoculated with 5 µL of a grown culture diluted to 10^6 cells/mL. Tubes containing nutrient broth plus 100 µL of sterile water or 100 µL of the respective solvent were seeded with the tested organisms as described above to serve as controls. After incubation, (12 h, 37 °C) absorbance of the grown culture was measured at 600 nm in a Perkin Elmer VDE 0871/B spectrometer (Überlingen, Germany). Growth reduction was assessed by the following equation:

$$\text{Growth reduction} = \frac{A_{\text{solvent}} - A_{\text{extract}}}{A_{\text{control}}} \times 100 \quad (1)$$

where A_{control} is the absorbance of the control grown culture, A_{solvent} is the absorbance of the culture incubated with solvent, and A_{extract} is the absorbance of the culture incubated with the tested extract.

Polyphenol content

Total phenolic content was determined with the Folin–Ciocalteu (FC) reagent (Fluka, Japan) [13]. Two hundred µL of the extract dissolved in methanol were mixed with 1 mL of FC reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 800 µL of Na_2CO_3 (60 g/L) (Sigma-Aldrich, Germany) solution were added to the mixture. After 90 min, the absorbance was measured at 765 nm. Results were expressed in gallic acid (Sigma-Aldrich, Germany) equivalents (mg GAE/L).

Antioxidant capacity

The radical scavenging activity was determined by using the stable DPPH radical (Sigma-Aldrich, Germany) [14]. The diluted extract sample (3.2 mL) was mixed with 800 µL of 400 µM DPPH in ethanol. The dilution depended on the concentration of each sample, and in all cases the final absorbance was in the correlation interval concentration–absorbance. After 30 min of incubation in the dark, the absorbance was measured at 520 nm. Antioxidant capacity was expressed in Trolox (Sigma-Aldrich, Germany) equivalents (mg TE/L).

HPLC–MS analysis

Crude extracts were analyzed as described by Rubilar et al. [15]. Samples were filtered through a 0.45 µm nylon filter, and injected (20 µL) into an HPLC–DAD system (Jasco UV-1575, Japan). A C18 Hypersil ODS column (250 mm × 4.6 mm, 5 µm particle size, Supelco) was used. Flow rate was set to 0.7 mL/min. Solvents used were 0.5% acetic acid–water solution (A) and methanol (B). Linearly ramping gradient was as follows: 0–10 min, 95A/5B; 10–60 min, 50A/50B; 60–80 min, 30A/70B; and 80–90 min, 95A/5B. Detection was performed at 280 nm. Three determinations were made on each sample. Equipment used for electrospray mass spectrometry in the positive ion mode was a HP-Serie 1100-MSD. Conditions were as follows: nitrogen as the drying gas at 13 L/min and

350 °C, nebulizer pressure at 40 psig and fragmentor voltage at 60 V.

Statistical analysis

All the measurements were done at least in triplicate and the results were expressed as mean ± standard deviation. Data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range. A correlation test was used to establish linear association between variables and responses significance. Significance of the results was established at $P < 0.05$.

Results and discussion

Polyphenol content and antioxidant capacity

The highest values of total phenols and antioxidant capacity corresponded to 50% ethanol/water extracts, both from leaves and fruits (Table 2). In general, the use of pure ethanol or pure water resulted in the lowest values of polyphenol content. Our previous work reported that polyphenols present in murta can be divided in two major groups [3]: (1) flavonol glycosides, mainly myricetin and quercetin glucoside, rhamnoside and dirhamnoside and, (2) to a minor extent, flavan-3-ol monomers, mainly epicatechin. The water solubility of flavonols increases with the degree of glycosidation, and, in general, the solubility of the monoglycosides increases with ethanol [16]. The solubility of epicatechin is also increased by using ethanol. This may explain that a combination of both solvents resulted in the highest polyphenol release. The polyphenol content of the extracts from fruits collected in the mountain was higher than the one in the valley, which was in turn higher than polyphenolic content of the coast fruit extracts. This confirms the fact that more extreme climatic conditions result in an increase of the polyphenols contained in plants, used as protective agents [10]. In leaves, however, there is no clear tendency and polyphenolic content depends more on the extraction solvent than on the plant

Table 3 Effects of murta fruit extracts on antimicrobial activity against *S. aureus* ATCC 25923

Microorganism	v/v[a]	Extracts from murta fruits Zone of inhibition (mm)		
		Mountain	Coast	Valley
S. aureus ATCC 25923	0/100	No effect	No effect	No effect
	25/75	0.7 ± 0.1	No effect	No effect
	50/50	1.8 ± 0.1[a]	1.4 ± 0.1[b]	1.6 ± 0.2[ab]
	75/25	1.0 ± 0.1	1.0 ± 0.2	1.2 ± 0.2
	100/0	1.1 ± 0.3	0.9 ± 0.3	1.0 ± 0.3

Different letters indicate significant differences among polyphenol content and antimicrobial activity of 50% ethanol/water extracts ($P < 0.05$)

[a] Volumetric water to ethanol ratio. Averages of at least three determinations ± SD

Table 2 Soluble solids (SS), polyphenol content (mg GAE/L), and antioxidant capacity (mg TE/L) of the extracts of leaves and fruit of murta (*Ugni molinae* Turcz.)

Source	Solvent[a] (v/v)	Leaves			Fruits		
		SS (%)	TE (mg/L)	GAE (mg/L)	SS (%)	TE (mg/L)	GAE (mg/L)
Mountain	0/100	9.5 ± 0.5	3,236 ± 67	3,270 ± 32	9.5 ± 0.8	4,089 ± 30	1,175 ± 7
	25/75	9.6 ± 0.7	4,985 ± 134	7,581 ± 32	9.3 ± 0.4	5,418 ± 15	41,92 ± 17
	50/50	9.6 ± 1.0	10,445 ± 100[a]	11,584 ± 43[b]	9.3 ± 0.3	5,801 ± 30[a]	40,37 ± 10[a]
	75/25	9.6 ± 0.4	6,427 ± 100	9,941 ± 0	9.4 ± 0.4	3,892 ± 23	2,430 ± 10
	100/0	9.4 ± 0.3	1,724 ± 134	5,946 ± 21	9.3 ± 0.3	3,270 ± 30	1,090 ± 13
Valley	0/100	9.7 ± 1.2	3,874 ± 100	2,900 ± 21	9.5 ± 0.5	1,191 ± 23	1,071 ± 13
	25/75	10.1 ± 0.8	3,378 ± 67	7,815 ± 43	9.0 ± 0.6	1,414 ± 23	3,754 ± 10
	50/50	10.1 ± 0.7	9,239 ± 67[b]	12,579 ± 43[a]	9.2 ± 0.6	3,025 ± 15[c]	3,823 ± 13[b]
	75/25	9.9 ± 0.5	8,483 ± 67	11,237 ± 21	9.2 ± 0.4	2,802 ± 30	1,957 ± 7
	100/0	9.9 ± 0.3	8,318 ± 100	7,868 ± 32	9.3 ± 0.2	1,212 ± 8	1,074 ± 10
Coast	0/100	9.9 ± 0.2	3,496 ± 100	1,996 ± 43	9.6 ± 0.2	2,042 ± 8	1,038 ± 13
	25/75	9.8 ± 0.6	7,727 ± 67	7,084 ± 32	8.8 ± 0.5	3,738 ± 30	3,669 ± 10
	50/50	9.9 ± 0.5	7,916 ± 134[c]	11,622 ± 32[b]	9.2 ± 0.7	4,137 ± 23[b]	3,210 ± 13[c]
	75/25	9.9 ± 0.4	7,632 ± 134	9,586 ± 32	9.3 ± 0.2	2,212 ± 23	1,490 ± 13
	100/0	9.6 ± 0.2	7,538 ± 134	6,443 ± 21	9.5 ± 0.2	3,020 ± 23	1,029 ± 13

Different letters indicate significant differences in polyphenol content and antioxidant capacity of 50% ethanol/water extracts ($P < 0.05$)

[a] Volumetric water to ethanol ratio. Averages of at least three determinations ± SD

Table 4 Effects of murta leaves extracts on antimicrobial activity against *S. aureus* ATCC 25923, *K. pneumoniae* ATCC 13883 and *P. aeruginosa* ATCC 27853

Microorganism	v/v[a]	Extracts from murta leaves Zone of inhibition (mm)		
		Mountain	Coast	Valley
S. aureus ATCC 25923	0/100	2.0 ± 0.1	1.2 ± 0.2	1.0 ± 0.8
	25/75	3.4 ± 1.3	3.2 ± 1.6	3.9 ± 0.7
	50/50	6.1 ± 0.9[a]	4.0 ± 1.4[b]	4.8 ± 1.0[b]
	75/25	5.4 ± 1.4	3.7 ± 1.5	4.3 ± 0.7
	100/0	5.4 ± 1.4	3.2 ± 1.5	4.1 ± 0.5
K. pneumoniae ATCC 13883	0/100	No effect	No effect	No effect
	25/75	1.5 ± 0.2	1.2 ± 0.4	1.1 ± 0.4
	50/50	2.3 ± 0.4[a]	1.4 ± 0.4[b]	2.1 ± 0.1[a]
	75/25	1.9 ± 0.5	1.1 ± 0.3	1.9 ± 0.2
	100/0	1.2 ± 0.2	1.0 ± 0.0	1.6 ± 0.0
P. aeruginosa ATCC 27853	0/100	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.6
	25/75	1.7 ± 0.4	2.1 ± 0.6	1.7 ± 0.6
	50/50	3.0 ± 0.4[a]	2.4 ± 0.7[b]	1.9 ± 0.6[b]
	75/25	2.5 ± 0.6	2.2 ± 0.4	1.7 ± 0.6
	100/0	1.7 ± 0.4	1.8 ± 0.2	1.5 ± 0.4

Different letters indicate significant differences among polyphenol content and antimicrobial activity of 50% ethanol/water extracts ($P < 0.05$)

[a] Volumetric water to ethanol ratio. Averages of at least three determinations ± SD

origin. It is possible that leaves, being perennial in opposite to fruits, use other different plant permanent components, able to stiffen their structure (e.g. lignins) to protect themselves against the adverse climatic factors [17]. Antioxidant capacity values were also higher for the fruits collected in the mountain, compared to the fruits from valley and coast. In general, however, the statistical analysis showed poor correlations between values of polyphenol content and antioxidant capacity ($P > 0.05$), being the exception the data corresponding to the extract of fruit from the mountain, with a linear regression coefficient of 0.843.

Antimicrobial activity

Extracts from both fruits and leaves showed antimicrobial activity against *S. aureus* (Tables 3, 4). In addition, leaf extracts were efficient against *K. pneumoniae* and *P. aeruginosa*. None of the extracts were active against growing of any of the fungi. Ethanol extracts showed either no antimicrobial activity or significantly lower compared to the aqueous extracts, which presumably means that the water-soluble compounds including polyphenols had the highest antimicrobial activity. Leaf extracts were also more active against *S. aureus* than the fruit extracts, likely ascribable to the higher content of phenols in the extract. ANOVA statistical analysis showed that the antimicrobial activity of leaf and fruit extracts was in general significantly affected by the extraction solvent, plant location and the combination of both factors. This also evidences the considerable influence of the location and the extraction solvent on the composition and properties of the extracts. Statistical analysis also showed significant linear correlations between the antimicrobial activity of leaf extracts and the polyphenol content. Independently of the plant material, the regression coefficient was higher than 0.700 for all the susceptible bacteria. Extracts from leaves of many plants have been previously demonstrated to have antibacterial activity. It could be hypothesized that some of these leaves, despite the species considered, share some of the active polyphenols. Leaves from *Alchornea cordifolia* and *Psidium guajava* Linn., for instance, were found to be active against *Staphylococcus*, and concretely on *S. aureus*. However, the compounds responsible for the antimicrobial effect have not been identified in these works [18, 19]. Murta extracts did not show antimicrobial activity against any of the probiotic bacteria evaluated. This confirms

Table 5 Effects of murta fruit extracts on antimicrobial activity against *S. aureus* ATCC 25923 in liquid media

Microorganism	v/v[a]	Extracts from murta fruits Growth reduction (%)		
		Mountain	Coast	Valley
S. aureus ATCC 25923	0/100	0	0	0
	25/75	6.5 ± 0.2	0	0
	50/50	8.0 ± 0.6	0	0
	75/25	2.0 ± 0.2	2.9 ± 1.4	10.8 ± 1.2
	100/0	1.3 ± 0.5	0	6.3 ± 1.8

[a] Volumetric water to ethanol ratio. Growth reduction from Eq. 1. Averages of at least three determinations ± SD

Table 6 Effects of murta leaves extracts on antimicrobial activity against *S. aureus* ATCC 25923, *K. pneumoniae* ATCC 13883 and *P. aeruginosa* ATCC 27853

Microorganism	v/v[a]	Extracts from murta leaves Growth reduction (%)		
		Mountain	Coast	Valley
S. aureus ATCC 25923	0/100	0	0	0
	25/75	0	9.3 ± 0.1	7.4 ± 2.8
	50/50	25.1 ± 2.2	30.4 ± 1.1	44.4 ± 10.7
	75/25	46.3 ± 3.9	31.2 ± 3.1	46.7 ± 1.1
	100/0	39.7 ± 8.6	26.6 ± 4.7	43.7 ± 16.3
K. pneumoniae ATCC 13883	0/100	0	0	0
	25/75	16.2 ± 3.7	0	5.2 ± 0.5
	50/50	23.6 ± 0.9	21.3 ± 4.9	20.0 ± 3.6
	75/25	22.7 ± 2.9	25.2 ± 5.9	26.5 ± 2.9
	100/0	18.5 ± 1.1	17.8 ± 7.4	22.2 ± 4.4
P. aeruginosa ATCC 27853	0/100	0	0.2 ± 0.3	0
	25/75	0	0	12.8 ± 1.4
	50/50	40.4 ± 8.4	41.2 ± 3.4	49.1 ± 0.8
	75/25	40.1 ± 6.6	40.0 ± 1.4	70.8 ± 12.1
	100/0	55.8 ± 10.9	53.4 ± 6.7	81.1 ± 3.8

S. aureus ATCC 25923, *K. pneumoniae* ATCC 13883 and *P. aeruginosa* ATCC 27853 in liquid media

[a] Volumetric water to ethanol ratio. Growth reduction from Eq. 1. Averages of at least three determinations ± SD

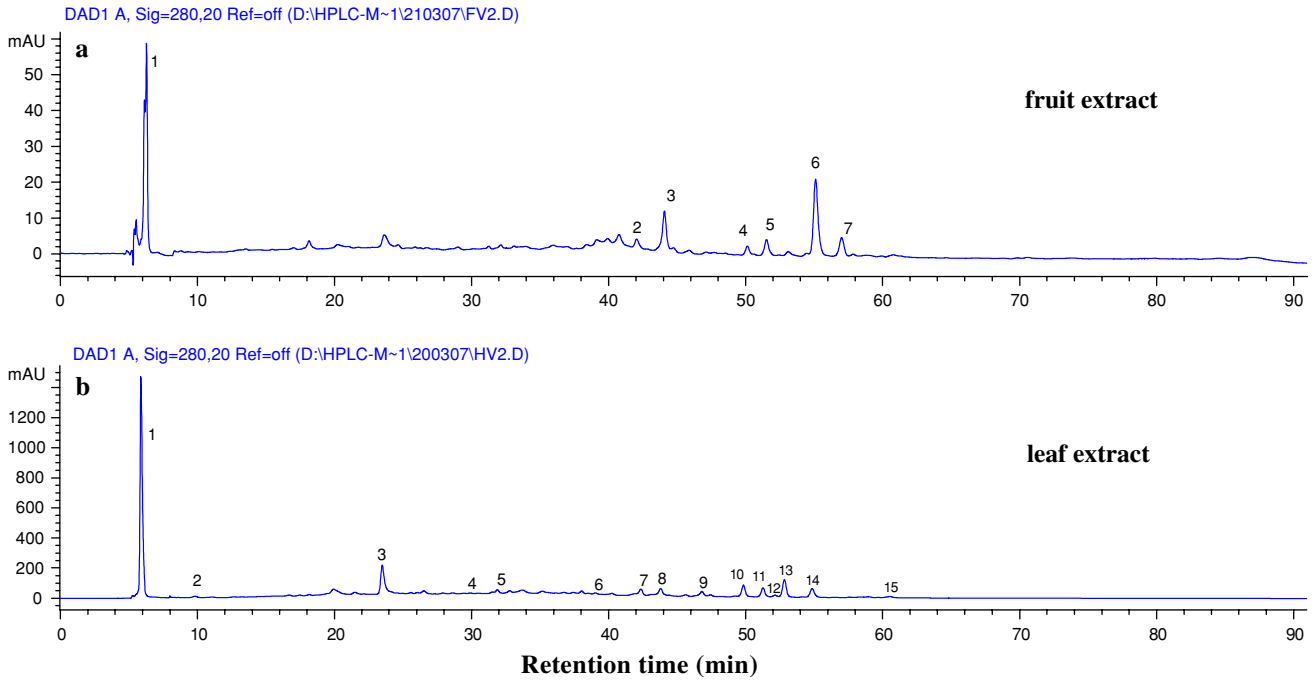

Fig. 1 Chromatograms of 50% ethanol/water extracts from fruits (4,037 mg GAE/l) and leaves (11,584 mg GAE/l) of mountain *Ugni molinae* Turcz ecotypes

murta extracts as suitable food additives in those functional foods in which additional probiotics are added (e.g. yoghourt, milk).

Antimicrobial activity compared to commercial antibiotics

The 50% ethanol/water extracts of leaves from the mountain (90 µL/disk) showed activity on *P. aeruginosa* and *K. pneumoniae* equivalent to 42 and 32% of that of the commercial antibiotic Gentamicine (10 µg), respectively. Likewise, the same mountain leaf extracts showed activity on *P. aeruginosa* and *K. pneumoniae* equivalent to 11 and 38% activity of the Penicillin G (10 IU), respectively. Considering the existing linear relationship (data not shown) between the extract volume on the disk and the antimicrobial activity, a higher amount of extract would be enough to equal the activity of the commercial antibiotics.

Table 7 Tentative identification of polyphenolic species in 50% water/ethanol extracts from fruits of murta of mountain origin

Peak	Retention time (min)	λ_{max} (nm)	(*m*/*z*)	Positive ion (*m*/*z*) fragments	Identification[a]
1	6.1	224–272	365		NI
2	42.1	276–348	303	(302 + 262) 564	NI
3	44.1	276–340	319	(318 + 162 + 23) 503	Myricetin glucoside
4	50.1	258–360	319	(318 + 162 + 23) 503	Myricetin glucoside
5	51.5	264–356	303	(302 + 146 + 146 + 23) 617	Quercetin dirhamnoside
6	55.1	256–356	303	(302 + 162 + 23) 487	Quercetin glucoside
7	57.0	256–354	303	(302 + 176 + 1) 479	Quercetin glucuronide

NI nonidentified

[a] The tentative identification of the monosaccharides linked to the phenolic species was supported by spectral data and published results on polyphenolic species present in murta

Table 8 Tentative identification of polyphenolic species in 50% water/ethanol extracts from leaves of murta of mountain origin

Peak	Retention time (min)	λ_{max} (nm)	(*m*/*z*)	Positive ion (*m*/*z*) fragments	Identification[a]
1	5.9	232–266	209, 315		NI
2	9.8	222–274	153, 345		NI
3	23.5	224–264	307, 471		Flavan-3-ol (E)GC
4	29.9	272	291	(290 + 1)	Flavan-3-ol (E)C
5	31.9	276	291	(290 + 1)	Flavan-3-ol (E)C
6	39.0	274	291, 425, 446, 1160		Polymeric flavan-3-ol (E)C
7	42.4	274–342	187, 335		NI
8	43.8	276–342	319, 481	(318 + 162 + 1) 481	Myricetin glucoside
9	46.8	264–358	303, 319, 315, 633	(318 + 146 + 146 + 23) 633 (302 + 146 + 162 + 23) 633	Myricetin and quercetin glucosides
10	49.8	258–360	319, 503	(318 + 162 + 23) 503	Myricetin glucoside
11	51.3	262–356	303, 617	(302 + 146 + 146 + 23) 617	Quercetin dirhamnoside
12	52.1	254–358	319, 451, 473	(318 + 132 + 1) 451 (318 + 132 + 23) 473	Myricetin xyloside
13	52.8	258–352	319, 487	(318 + 146 + 23) 487	Myricetin rhamnoside
14	54.9	256–356	303, 487	(302 + 162 + 23) 487	Quercetin glucoside
15	60.5	256–350	303, 471	(302 + 146 + 23) 471	Quercetin rhamnoside

(E)GC (epi)gallocatechin, *(E)C* (epi)catechin; *NI* nonidentified

[a] The tentative identification of the monosaccharides linked to the phenolic species was supported by spectral data and published results on polyphenolic species present in Murta

Effect of extracts on bacterial growth in liquid media

A decrease in the growing of *S. aureus* (for both fruit and leaf extracts) and in *K. pneumoniae* and *P. aeruginosa* (for leaf extracts) was observed when 100 µL of extracts were added to 2 mL of liquid medium (Tables 5, 6). Fruit extracts showed no activity on the last two bacterial species. Higher extract concentrations were not evaluated due to the precipitation of the components in the culture media. Precipitation was attributed to the formation of polyphenol–protein complexes. Previous works have indeed suggested the need of finding low-protein culture media for the evaluation of the antimicrobial activity of extracts rich in polyphenols [20]. ANOVA statistical analysis showed the significance of the plant location, extraction solvent, and the combination of both factors on the growth inhibition of *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. As observed in the solid culture media, ethanolic extracts did not exert any antimicrobial activity. These results confirm again that only the water-soluble extracts from murta are able to prevent the growing of undesirable bacteria.

Tentative identification of active polyphenols

Extracts of fruits and leaves from the mountain area, obtained by a 50% ethanol/50% water mixture, were found to exert the highest antioxidant and antimicrobial activity. Myricetin glucoside, quercetin glucoside, quercetin

glucuronide and quercetin dirhamnoside were the only polyphenols identified in the extract of mountain fruit (Fig. 1a; Table 7). The extract from leaves showed a higher variety of polyphenolic compounds (Fig. 1b; Table 8). Along with the ones found in the fruit, leaves were also found to contain other flavonol glycosides like myricetin xyloside and myricetin rhamnoside (Fig. 2). The main difference in the polyphenolic profile of leaves and fruits was the presence of flavan-3-ols, both in monomeric and in polymeric form, in the extracts from the leaves. Flavan-3-ols in polymeric form were detected for the first time in murta extracts. In our previous work about identification of murta extracts, simple phenolic acids like gallic acid were only detected in aqueous extracts, indicating the major influence of the extractant solvent on the released species [3]. Likewise, flavonol glycosides and flavan-3-ols were exclusively extracted by alcohols. The occurrence of additional polyphenolic species in leaves may explain the higher antimicrobial activity of the extracts, and also the inhibitory effect on some bacteria on which the fruit extracts do not show any activity. The antimicrobial activity of myricetin and quercetin derivates extracted from fruits has been previously reported [21, 22]. It is also possible that flavonol glycosides act in synergy with other polyphenols to boost their antimicrobial activity [7, 8, 23, 24]. Rauha et al. [22] have made one of the most complete

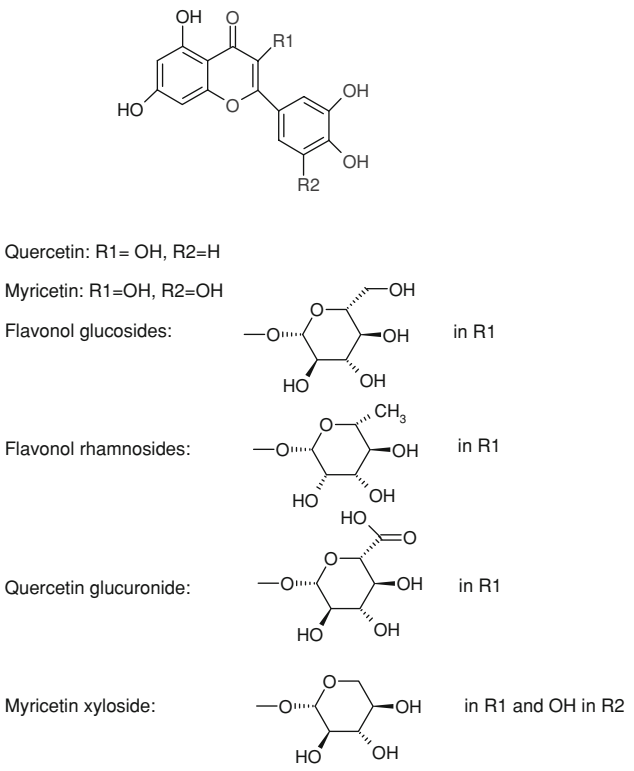

Fig. 2 Chemical structure of flavonol glycosides detected in murta leaves and fruits

studies about antimicrobial effect of polyphenols and found that quercetin and kaempferol, both in monomeric form or combined with sugars, were active against several harmful bacteria. They have also reported that catechin does not show any antibacterial effect; however, the antimicrobial action of other flavan-3-ols with higher degree of polymerization has not been evaluated yet. Besides polyphenols, other compounds present in murta could also have a degree of responsibility on the antimicrobial effect. However, data from literature about other vegetal species make hypothesize that phenol compounds are likely the main responsible for the antimicrobial activity.

In conclusion, it could be said that the climatic conditions at which the plant is subjected during cultivation is the first factor conditioning the polyphenolic profile of murta fruits and leaves. Extreme climatic conditions may promote an increase in the polyphenol content of leaves, being the extracts more concentrated and active against harmful bacteria. Extraction procedure is the other factor influencing the extracts composition. Even if flavonol glycosides—main polyphenol components of murta leaves and fruits—are soluble in water, ethanol can help increase the solubility of non/low glycosylated flavonols and flavan-3-ol monomers. Extracts of murta leaves were particularly active against the growing of *S. aureus*, *P. aeruginosa* and *K. pneumoniae*, both in solid or in liquid phase. Only water-soluble compounds may exert antimicrobial activity. The phenolic composition, and in turn the plant location and extraction procedure which influenced the polyphenolic content, conditioned significantly the antimicrobial effect of leaf murta extracts. Extracts from mountain were the most active again. Fruit extracts were not as active as leaf extracts, due to the lower level of compounds with antimicrobial activity, i.e. flavan-3-ols and flavonol glycosides, and other compounds extracted that may have antimicrobial activity too (e.g. acids or small lignin fragments).

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