Infusion and decoction of wild German chamomile: bioactivity and characterization of organic acids and phenolic compounds

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Abstract

Natural products represent a rich source of biologically active compounds and are an example of molecular diversity, with recognized potential in drug discovery. Herein, methanol extract of *Matricaria recutita* L. (German chamomile) and its decoction and infusion (the most consumed preparations of this herb) were submitted to an analysis of phytochemicals and bioactivity evaluation. The antioxidant activity was determined by free radicals scavenging activity, reducing power and inhibition of lipid peroxidation; the antitumour potential was tested in human tumour cell lines (breast, lung, colon, cervical and hepatocellular carcinomas), and the hepatotoxicity was evaluated using a porcine liver primary cell culture (non-tumour cells). All the samples revealed antioxidant properties. Decoction had no antitumour activity (GI_{50}>400 μg/mL) which could indicate that this bioactivity might be related to compounds (including phenolic compounds) that were not extracted or that were affected by the decoction procedure. Both plant methanol extract and infusion showed inhibitory activity of the growth of HCT-15 (GI_{50} 250.24 and 298.23 μg/mL, respectively) and HeLa (GI_{50} 259.36 and 277.67 μg/mL, respectively) cell lines, without hepatotoxicity (GI_{50}>400 μg/mL). Infusion and decoction gave higher contents of organic acids (24.42 and 23.35 g/100 g dw). Otherwise, the plant methanol extract presented the highest amounts of phenolic acids (3.99 g/100 g dw) and flavonoids (2.59 g/100 g dw). The major compound found in all the preparations was luteolin *O*-acylhexoside. Overall, German chamomile contains important phytochemicals with bioactive properties (mainly antitumour potential selective to colon and cervical carcinoma cell lines) to be explored in the medicine, food, and cosmetic industries.

Keywords: Matricaria recutita; German chamomile; Antioxidant activity; Antitumour potential; Organic acids; Phenolic compounds

1. Introdution

German chamomile (*Matricaria recutita* L.) is an annual herbaceous flowering plant native to Europe. This herb has been used as herbal remedies for thousands of years (Crevin, 1990). It has been used traditionally as a medicinal and pharmaceutical preparation, due to its anti-inflammatory, anti-spasmodic, analgesic, antibacterial, anti-allergic antioxidant and mild astringent properties, and healing medicine (Maschi et al., 2008; McKay, & Blumberg, 2006; Weiss, 1988). Externally, chamomile has been used to treat diaper rash, cracked nipples, chicken pox, ear and eye infections, disorders of the eyes including blocked tear ducts, conjunctivitis, nasal inflammation and poison ivy (Srivastava, Shankar, & Gupta, 2010).

The use of German chamomile teas as medicinal preparations has a long tradition in various countries. Infusions and essential oils are used in a number of commercial products including soaps, detergents, perfumes, lotions, ointments, hair products, baked goods, confections, alcoholic beverages and herbal teas (Gupta, Mittal, Bansal, Khokra, & Kaushik, 2010). Traditionally, chamomile flowers are prepared as an infusion with water, to make an herbal tea (Harbourne, Jacquier, & O'Riordan, 2009). Recent research supports this use and shows that these properties are partly due to the phenolic content (Maschi et al., 2008; McKay, & Blumberg, 2006).

German chamomile contains several classes of biologically active compounds including essential oils (Granzera, Schneider, & Stuppner, 2006; Petronilho, Maraschin, Coimbra, & Rocha, 2012) and several polyphenols (McKay, & Blumberg, 2006; Nováková, Vildová, Mateus, Gonçalves, & Solich, 2010). Some phenolic compounds have the capacity to quench lipid peroxidation products, prevent DNA oxidative damage, and scavenge reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals (Kahkonen et al., 1999). Flavonoids are the most abundant phenolic

compounds in herbs (Mladěnka et al., 2010) and their effects on human health are very often ascribed to their potential ability to act diminishing free radical steady state concentration in biological systems providing antioxidant protection (Galleano, Verstraeten, Oteiza, & Fraga, 2010). Such ability could be possible considering that polyphenols have chemical structures supporting the scavenging of free radicals and the chelation of redox-active metals. In parallel, it has been reported that certain flavonoids can provide benefits in pathological situations associated with high free radical production, (e.g. hypertension and cardiovascular disease) (Galleano et al., 2010).

Some related studies dealing with *M. recutita* flowers are available in literature, including reports on antioxidant properties of its methanol extract (Barros, Oliveira, Carvalho, & Ferreira, 2010; Miliauskas, Venskutonis, & Beek, 2004), antitumour potential of aqueous and organic extracts (Strivastava & Gupta, 2007; Strivastava & Gupta, 2009), and phenolic composition of methanolic extracts (Mulinacci, Romani, Pinelli, Vincieri, & Prucher, 2000; Nováková et al., 2010). Nevertheless, studies on *M. recutita* infusion and decoction, the most consumed preparation of this herb, are scarce, and therefore, the present study reports the bioactive properties (antioxidant and antitumour activities, and hepatotoxicity), organic acids and phenolic characterization of wild *M. recutita* infusions and decoction (the most consumed preparations of this herb).

2. Materials and methods

2.1. Standards and reagents

Acetonitrile 99.9% was of HPLC grade from Fisher Scientific (Lisbon, Portugal). The phenolic compound standards (chlorogenic acid; ferulic acid, luteolin-6-*C*-glucoside; luteolin-7-*O*-glucoside; myricetin; protocatechuic acid and quercetin 3-*O*-glucoside) were from Extrasynthese (Genay, France). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was

obtained from Alfa Aesar (Ward Hill, MA, USA). Fetal bovine serum (FBS), L-glutamine, Hank's balanced salt solution (HBSS), trypsin-EDTA (ethylenediaminetetraacetic acid), nonessential amino acids solution (2 mM), penicillin/streptomycin solution (100 U/mL and 100 mg/mL, respectively), RPMI-1640 and DMEM media were from Hyclone (Logan, USA). Acetic acid, ellipticine, sulforhodamine B (SRB), trypan blue, trichloroacetic acid (TCA), Tris and all organic acids standards (L-ascorbic acid; citric acid, fumaric acid, malic acid, shikimic acid; succinic acid; oxalic acid and quinic acid) were from Sigma Chemical Co. (Saint Louis, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.2. Sample

Matricaria recutita flower heads and leafy flowering stems of about 15 cm long were collected in 2009, in late spring and early summer, in the Natural Park of Montesinho territory, Trás-os-Montes, North-eastern Portugal. Morphological key characters from Rothmaler (2007) were used for plant identification. Voucher specimens are deposited in the Escola Superior Agrária de Bragança herbarium (BRESA). The sample was lyophilized (FreeZone 4.5, Labconco, Kansas, USA), reduced to a fine dried powder (20 mesh) and mixed to obtain homogenate sample.

2.3. Infusion, decoction and methanol extract preparation

For infusion preparation, the sample (1 g of lyophilized flowers and leafy flowering stems) was added to 200 mL of boiling distilled water and left to stand at room temperature for 5 min, and then filtered under reduced pressure. The obtained infusion was frozen and lyophilized.

For decoction preparation, the sample (1 g) was added to 200 mL of distilled water, heated (heating plate, VELP scientific) and boiled for 5 min. The mixture was left to stand at room temperature for 5 min more, and then filtered under reduced pressure. The obtained decoction was frozen and lyophilized.

A methanol extract was also obtained from the lyophilized plant material, and used as control. The sample (1 g) was extracted by stirring with 25 mL of methanol (25 ºC at 150 rpm) for 1 h and subsequently filtered through Whatman No. 4 paper. The residue was then extracted with 25 mL of methanol (25 ºC at 150 rpm) for 1 h. The combined methanolic extracts were evaporated at 40 ºC (rotary evaporator Büchi R-210) to dryness.

2.4. Evaluation of bioactivity

2.4.1. Antioxidant activity. The lyophilized infusion and decoction, and the plant methanol extract were redissolved in water (final concentration 2.5 mg/mL); the final solution was further diluted to different concentrations to be submitted to antioxidant activity evaluation by *in vitro* assays. DPPH radical-scavenging activity was evaluated by using a ELX800 microplate Reader (Bio-Tek Instruments, Inc; Winooski, USA), and calculated as a percentage of DPPH discolouration using the formula: $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution (Pereira, Barros, Martins, & Ferreira, 2012). Reducing power was evaluated by the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm in the microplate Reader mentioned above (Pereira et al., 2012). Inhibition of β-carotene bleaching was evaluated though the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which is measured by the formula: β-carotene absorbance after 2h of

assay/initial absorbance) × 100 (Pereira et al., 2012). Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decreasing in thiobarbituric acid reactive substances (TBARS); the colour intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the following formula: [(A - B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively (Pereira et al., 2012). The results were expressed in EC_{50} value (sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay). Trolox was used as positive control.

2.4.2. Antitumour activity

The lyophilized infusion and decoction, and the plant methanol extract were redissolved in water (final concentration 8 mg/mL); the final solution was further diluted to different concentrations to be submitted to antitumour activity *in vitro* evaluation. Five human tumour cell lines were used: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HCT-15 (colon carcinoma), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma). Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium containing 10% heat-inactivated FBS (MCF-7, NCI-H460 and HCT-15) and 2 mM glutamine or in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin (HeLa and HepG2 cells), at 37 ºC, in a humidified air incubator containing 5% CO_2. Each cell line was plated at an appropriate density (7.5×10^3 cells/well for MCF-7, NCI-H460 and HCT-15 or 1.0×10^4 cells/well for HeLa and HepG2) in 96-well plates and allowed to attach for 24 h. Cells were then treated for 48 h with the different diluted sample solutions. Following this incubation period, the adherent cells were fixed by adding cold 10%

trichloroacetic acid (TCA, 100 µL) and incubated for 60 min at 4 ºC. Plates were then washed with deionized water and dried; sulforhodamine B solution (0.1% in 1% acetic acid, 100 µL) was then added to each plate well and incubated for 30 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air dried, the bound SRB was solubilised with 10 mM Tris (200 µL, pH 7.4) and the absorbance was measured at 540 nm (Monks et al., 1991) in the microplate reader mentioned above. The results were expressed in GI_{50} values (sample concentration that inhibited 50% of the net cell growth). Ellipticine was used as positive control.

2.4.3. Hepatotoxicity

A cell culture was prepared from a freshly harvested porcine liver obtained from a local slaughter house, and it was designed as PLP2. Briefly, the liver tissues were rinsed in Hank's balanced salt solution containing 100 U/mL penicillin, 100 µg/mL streptomycin and divided into 1×1 mm^3 explants. Some of these explants were placed in 25 cm^2 tissue flasks in DMEM medium supplemented with 10% fetal bovine serum, 2 mM nonessential amino acids and 100 U/mL penicillin, 100 mg/mL streptomycin and incubated at 37 ºC with a humidified atmosphere containing 5% CO_2. The medium was changed every two days. Cultivation of the cells was continued with direct monitoring every two to three days using a phase contrast microscope. Before confluence, cells were subcultured and plated in 96-well plates at a density of 1.0×10^4 cells/well, and cultivated in DMEM medium with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin (Abreu et al., 2011). Cells were treated for 48 h with the different diluted sample solutions and the same procedure described in the previous section for SRB assay was followed. The results were expressed in GI_{50} values (sample concentration that inhibited 50% of the net cell growth). Ellipticine was used as positive control.

2.5. Analysis of organic acids

Organic acids were determined following a procedure previously optimized and described by the authors (Barros, Pereira, & Ferreira, 2012). Analysis was performed by ultra fast liquid chromatograph (UFLC) coupled to photodiode array detector (PDA), using a Shimadzu 20A series UFLC (Shimadzu Cooperation). Detection was carried out in a PDA, using 215 nm and 245 as preferred wavelengths. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight (dw).

2.6. Analysis of phenolic compounds

Phenolic compounds were determined by HPLC (Hewlett-Packard 1100, Agilent Technologies, Santa Clara, USA) as previously described by the authors (Barros et al., 2012). Double online detection was carried out in the diode array detector (DAD) using 280 nm and 370 nm as preferred wavelengths and in a mass spectrometer (MS) connected to the HPLC system via the DAD cell outlet. The phenolic compounds were characterized according to their UV and mass spectra and retention times, and comparison with authentic standards when available. For quantitative analysis, calibration curves were prepared from different standard compounds. The results were expressed in g per 100 g of dry weight (dw).

2.7 Statistical analysis.

All the assays were carried out in triplicate in three different samples of infusion, decoction and plant methanol extract; the results of bioactivity (antioxidant and

antitumour activities, and hepatotoxicity) and of organic acids and phenolic compounds composition are expressed as mean values±standard deviation (SD). The statistical differences represented by letters were obtained through one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with $\alpha = 0.05$. These treatments were carried out using SPSS v. 18.0 program.

3. Results and Discussion

3.1. Evaluation of bioactivity

The antioxidant activity was determined by free radicals scavenging activity, reducing power and inhibition of lipid peroxidation in brain cell homogenates, the antitumour potential was tested in human tumour cell lines (breast, lung, colon, cervical and hepatocellular carcinomas), and the hepatotoxicity was evaluated using a porcine liver primary cell culture. The results are shown in **Table 1**.

The infusion and decoction lyophilized samples gave similar antioxidant activity properties and were better than the plant methanol extract in the radical scavenging assays (DPPH and β-carotene bleaching inhibition). Otherwise, the plant methanol extract gave higher reducing power and lipid peroxidation inhibition measured by TBARS assay (lowest EC_{50} values). The mechanisms involved in the assays used to evaluate antioxidant activity are different and, therefore, each plant preparation can have different compounds with specific capacities to participate in those mechanisms.

Trolox and ellipticine were used as positive controls of antioxidant and antitumour activities evaluation assays, but should not be considered as standards and the comparison with extracts/oral preparations results should be avoided, because they are individual compounds and not mixtures.

Regarding antitumour effects, *M. recutita* infusion and plant methanol extract showed to be selective for HCT-15 and HeLa, since no activity was observed against the other cell lines: MCF-7, NCI-H460 and HepG2. Nevertheless, none of the *M. recutita* preparations showed hepatotoxicity in the porcine liver primary cell culture (non-tumour cells; PLP2) (**Table 1**). The plant methanol extract was slightly more potent than the infusion sample in HCT-15 and HeLa human cell lines. Decoction preparation had no antitumour effects at the maximal concentration used (400 µg/mL). The results obtained are in agreement with other authors that reported minimal growth inhibitory effects in normal cells, but a significant reduction in cell viability in various human cancer cell lines, mainly from methanolic fractions rather than aqueous ones (Srivastava & Gupta, 2007, 2009). The absence of antitumour activity in decoction of another chamomile species, *Chamaemelum nobile* (Roman chamomile), was previously reported by us (Guimarães et al., 2012).

It should be highlighted that *M. recutita* has been included in commercial mixtures for different pharmacological applications such as i) TBS-101 (a mixture of seven standardized botanical extracts) that showed an outstanding safety profile with significant anticancer activity against androgen-refractory human prostate cancer PC-3 cells, both *in vitro* and *in vivo* (Evans, Dizeyi, Abrahamsson, & Persson, 2009); ii) STW 5 (a mixture of nine standardized botanical extracts) for treatment of gastrointestinal disorders, with a mechanism of action related to their antioxidant properties (Schempp, Schempp, Weiser, Kelber, & Elstner, 2006). Nevertheless, chemical characterization and bioactivity evaluation of *M. recutita* infusion and decoction, the most consumed preparations of this herb, have been discarded being addressed herein.

3.2. Analysis of organic acids and phenolic compounds

Oxalic, quinic, malic, citric and succinic acids were quantified in all the extracts of *M. recutita*, malic acid being the most abundant organic acid (**Table 2**). Shikimic and fumaric acids were present in low or vestigial amounts. Ascorbic acid was not detected neither in infusion nor decoction, and was present in traces in the plant extract. Infusion and decoction gave similar contents in organic acids, and higher than the plant extract.

Organic acids might have antioxidant properties, such is the case of citric and oxalic acids (Hraš, Halodin, Knez, & Bauman, 2000; Kayashima & Katayama, 2002), which may contribute for the antioxidant activity of the samples studied herein.

The main phenolic compounds found in *M. recutita* plant and in its decoction and infusion were phenolic acids and derivatives, as also flavonoids such as flavonols and flavones (**Tables 3** and **4**). The chromatographic profile of the three plant preparations can be observed in **Figure 1**. Up to twenty phenolic compounds, including phenolic acids and flavonoids were detected in the *M. recutita* preparations (**Table 3**).

Compound 2 was identified as protocatechuic acid by comparison of its UV spectrum and retention time with a commercial standard. Thirteen hydroxycinnamic acid derivatives (peaks 1, 3-9, 11, 12, 14, 16 and 19) were detected, ten being quinic acid derivatives (1, 3-6; 9, 12, 14, 16 and 19), whose identities were assigned based on their MS spectra and fragmentation patterns. These compounds released characteristic MS^2 fragment ions at *m/z* 191 (deprotonated quinic acid), 179 (deprotonated caffeic acid) or 193 (deprotonated ferulic acid), which together with their pseudo molecular ions $[M-H]^-$ at *m/z* 353, 515, 677 or 367 allowed their identification as quinic acid derivatives containing one, two or three caffeic acid moieties or one ferulic acid moiety, respectively. The assignments of the different caffeoylquinic acid isomers were made using the recommended IUPAC numbering system, as also the hierarchical keys

previously developed by Clifford, Johnston, Knight, & Kuhnert (2003) and Clifford, Knight, & Kuhnert (2005).

Compound 6 was positively identified as 5-*O*-caffeoylquinic acid by comparison with an authentic standard, as also to its MS fragmentation pattern. Compound 5 was tentatively assigned as the corresponding *cis* isomer of 5-*O*-caffeoylquinic acid based on its fragmentation pattern and lower levels compared with peak 6. Furthermore, hydroxycinnamoyl *cis* derivatives would be expected to elute before the corresponding *trans* ones, as observed after UV irradiation (366 nm, 24 h) of hydroxycinnamic acids in our laboratory.

Compound 1 ($[M-H]^-$ at *m/z* 353) was identified as 3-*O*-caffeoylquinic acid, yielding the base peak at *m/z* 191 and the ion at *m/z* 179 with an intensity >55% base peak, characteristic of 3-acylchlorogenic acids as reported by Clifford et al. (2003, 2005). Compound 4 was easily distinguished from the other two isomers by its base peak at *m/z* 173 [quinic acid-H-H_2O]$^-$, accompanied by a secondary fragment ion at *m/z* 179 with approximately 84% abundance of base peak, which allowed identifying it as 4-*O*-caffeoylquinic acid according to the fragmentation pattern described by Clifford et al. (2003, 2005). Compound 9 was tentatively identified as 5-*O*-feruloylquinic acid taking into account its fragmentation pattern and relative intensities similar to 5-*O*-caffeoylquinic acid.

Compounds 3, 14, 16 and 19 ($[M-H]^-$ at *m/z* 515) could be assigned as dicaffeoylquinic acids and were assigned to 1,5-*O*-, 3,4-*O*-, 3,5-*O*- and 4,5-*O*- dicaffeoylquinic acids, respectively, based on their elution order, fragmentation pattern and relative abundances (Clifford et al., 2003, 2005). MS^2 fragmentation of compound 14 yielded the formation of signals corresponding to "dehydrated" fragment ions at *m/z* 335 [caffeoylquinic acid –H-H_2O]$^-$ and *m/z* 173 [quinic acid-H-H_2O]$^-$, characteristic of 4-acyl-caffeoylquinic

acids. Furthermore, according to Clifford et al. (2005), the intensity of signal at *m/z* 335 (25% of base peak) is more intense than in 4,5-*O*-dicaffeoylquinic (barely detectable, 4% of base peak). These observations allowed assigning compound 14 as 3,4-*O*-dicaffeoylquinic acid. The fragmentation pattern for 3,5-*O*-dicaffeoylquinic (compound 16) acid was similar to the one previously reported by Clifford et al. (2005). MS^2 base peak was at *m/z* 353, produced by the loss of one of the caffeoyl moieties [M-H-caffeoyl]$^-$, and subsequent fragmentation of this ion yielded the same fragments as 5-caffeoylquinic acid at *m/z* 191, 179 and 135, although in this case with a comparatively more intense signal at *m/z* 179 [caffeic acid-H]$^-$ (75% base peak). Compound 19 was assigned to 4,5-*O*- dicaffeoylquinic acid since its fragmentation was identical to those previously reported by Clifford et al. (2005). In this case, the signal at *m/z* 335 was barely detectable (<5 % of base peak) and the intense signal at *m/z* 173, is characteristic for an isomer substituted at position 4, which indicated that whereas 3,4-*O*-dicaffeoylquinic acid initially loses the caffeoyl moiety at position 3, in the case of 4,5-*O*-dicaffeoylquinic acid would initially lose that at position 5. Compound 3 was assigned as 1,5-*O*-dicaffeoylquinic acid, following the criteria the reported by Clifford et al. (2005), the weak ions at *m/z* 335 and 179 (<10 % of base peak).

Compound 12 was identified as tricaffeoylquinic acid according to its pseudomolecular ion [M-H]$^-$ at *m/z* 677 and diagnostic MS^2 fragments at *m/z* 515 (loss of the first caffeoyl), *m/z* 353 (loss of the second caffeoyl), *m/z* 191 (loss of the third caffeoyl to give quinic acid ion), *m/z* 179 (caffeic acid ion). The signal observed at *m/z* 497 can be interpreted by the loss of a water molecule from the ion at *m/z* 515. According to the relative intensities of different tricaffeoylquinic acid isomers reported by Lin & Harnly (2008), this compound could be assigned as 1,3,5-*O*-tricaffeoylquinic acid or 1,4,5-*O*-tricaffeoylquinic acid.

Compounds 7 and 8 with MS^2 fragments at *m/z* 193 ([ferulic acid-H]$^-$) resulting from the loss of a hexosyl moiety, -162 mu, and 176 ([ferulic acid-H-H_2O]$^-$) was tentatively assigned as a ferulic acid hexoside. Compound 7 presented similar UV and mass spectra characteristics as peak 8 but an earlier retention time, taking into account the observation above it was tentatively identified as *cis* ferulic acid hexoside. Similarly, compound 11 was tentatively identified as ferulic acid hexoside dimer.

Flavones were the most abundant flavonoids present in the samples analysed (**Tables 3** and **4**).

Compounds 13, 15, 18 and 20 were identified as luteolin derivatives. Compounds 15, 18 and 20 presented a pseudomolecular ions [M-H]- at *m/z* 447 and 489 releasing a common MS^2 fragment at *m/z* 285 ([M-H-162]$^-$ and [M-H-42-162]$^-$, loss of hexosyl and acetylhexoside moieties, respectively). They were tentatively identified as luteolin 7-*O*-glucoside (retention time compared with a commercial standard), and two luteolin *O*-acetylhexoside (compound 18 and 20), respectively. Compound 13 presented a pseudomolecular ion [M-H]$^-$ at *m/z* 651 releasing three MS^2 fragments at *m/z* 489, 447 and 285 (loss of hexosyl, acetylhexosyl and acetyldihexosyl moieties, respectively), being tentatively identified as luteolin acetylhexoside hexoside.

Flavonols (compounds 10 and 17) were also found in the studied samples (**Table 3**). Compound 10 presented a pseudomolecular ion [M-H]$^-$ at *m/z* 479, releasing an MS^2 fragment at *m/z* 317 ([M-H-162]$^-$, loss of an hexosyl moiety), corresponding to myricetin, which allowed a tentative identification of the compound as myricetin *O*-hexoside. Compound 17 corresponded to a quercetin derivative presenting a pseudomolecular ion [M-H]$^-$ at *m/z* 505 releasing an MS^2 fragment at *m/z* 301 (quercetin; [M-H-42-162]$^-$, loss of an acetylhexoside moiety). It is known that the introduction of a glycoside on the hydroxyls at positions 7, 3′ or 4′ has no effect on

wavelength maxima or the spectrum shape in relation to the aglycone (Mabry, Markham, & Thomas, 1970). Thus, quercetin 7-*O*-glycosides would have λ_{max} in Band I around 370 nm, while quercetin 3 *O*-glycosides are hypsochromically shifted to around 354 nm. Since this compound presented λ_{max} at 372 nm it was tentatively identified as quercetin 7-*O*-acetylhexoside.

The amounts of the phenolic compounds found varied among the different preparations and flavonols decreased in infusions and decoctions, but otherwise *cis* isomer of caffeoylquinic and ferulic acids derivatives appeared in these preparations (**Table 4** and **Figure 1**). The plant methanol extract (control) presented the highest amounts of phenolic acids (3.99 g/100 g dw) and flavonoids (2.59 g/100 g dw) as also total phenolic compounds (6.58 g/100 g dw), followed by infusion (5.00 g/100 g dw) and decoctions (3.51 g/100 g dw). The same was observed in *Chamaemelum nobile* (Roman chamomile) in a previously work of our research group (Guimarães et al., 2012).

The major compound found in the herbal plant and in the preparations was luteolin *O*-acylhexoside (compound 20). Mulinacci et al. (2000), Nováková et al. (2010), Harbourne et al., (2009) and Srivastava & Gupta, (2009) reported the presence of apigenin 7-*O*-glucoside and other apigenin derivatives, but these compounds were not detected in our samples.

Furthermore, Mulinacci et al. (2000) revealed the presence of different flavonoids, such as patuletin and other quercetin derivatives. In relation to the quantification, no comparison can be made, due to the fact that those authors only presented percent area measured at 335 nm of the main phenolic compounds found.

Nováková et al. (2010) presented the phenolic profile of methanolic extracts of *M. recutita* herbal flowers and infusions, demonstrating also lower values in infusions preparation when compared to the herbal plant methanolic extract. These authors also

found a dissimilar profile than the one obtained in this study, finding other flavonoids, such as kaempferol, isorhamnetin and different quercetin derivatives in their samples. The quantification was expressed in μmol/L, being difficult to compare with the results of *M. recutita* obtained herein.

Overall, it was observed a decrease in the amount of phenolic compounds in the plant infusion or decoction, compared to the methanol extract; the same was not observed for organic acids, which indicates that these compounds are better extracted with hot water than with methanol. The decoction had no antitumour effects which could indicate that these effects might be related to compounds (including phenolic compounds) that were not extracted or that were affected by the decoction procedure. Both plant methanol extract and infusion showed inhibitory activity of the growth of colon and cervical carcinoma cell lines, without toxicity for hepatocyte normal cells. Therefore, wild German chamomile (*M. recutita*) may be considered a source of important phytochemicals with bioactive properties to be explored in the medicine, food, and cosmetic industries.

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Table 1. Antioxidant and antitumour activities, and hepatotoxicity of wild *Matricaria recutita* (mean ± SD).

	Infusion	Decoction	Plant methanol extract*	Positive control**
Extraction yield (%)	16.25 ± 0.59	19.37 ± 1.09	16.09 ± 0.77	-
Antioxidant activity (EC_{50} values, µg/mL)				
DPPH scavenging activity	394.97 ± 44.31[b]	344.02 ± 18.65[b]	800.36 ± 49.09[a]	43.03 ± 1.71
Reducing power	316.61 ± 2.46[a]	318.75 ± 3.01[a]	232.49 ± 26.19[b]	29.62 ± 3.15
β-carotene bleaching inhibition	422.72 ± 92.91[b]	497.34 ± 107.67[b]	661.11 ± 21.93[a]	2.63 ± 0.14
TBARS inhibition	511.01 ± 17.28[a]	508.44 ± 4.43[a]	183.48 ± 3.52[b]	3.73 ± 1.90
Antitumour activity (GI_{50} values, µg/mL)	Infusion	Decoction	Plant methanol extract	Positive control**
MCF-7 (breast carcinoma)	>400	>400	>400	0.91±0.04
NCI-H460 (non-small lung cancer)	>400	>400	>400	1.42±0.00
HCT-15 (colon carcinoma)	298.23±11.58[a]	>400	250.24±18.38[b]	1.91±0.06
HeLa (cervical carcinoma)	277.67±9.04[a]	>400	259.36±7.57[b]	1.14±0.21
HepG2 (hepatocellular carcinoma)	>400	>400	>400	3.22±0.67
Hepatotoxicity (GI_{50} value, µg/mL)				
PLP2	>400	>400	>400	2.06±0.03

*Results reported in Barros et al., 2010. **Trolox and ellipticine for antioxidant and antitumour activity assays, respectively. EC_{50} values correspond to the sample concentration achieving 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in human tumour cell lines or in liver primary culture PLP2. In each row different letters mean significant differences ($p<0.05$).

Table 2. Identification and quantification of organic acids in wild *Matricaria recutita* (mean ± SD).

Organic acid	Infusion	Decoction	Plant (control)
Oxalic acid	8.45 ± 0.32[a]	8.60 ± 0.47[a]	3.24 ± 0.05[b]
Quinic acid	0.24 ± 0.00[b]	0.88 ± 0.19[a]	0.17 ± 0.00[c]
Malic acid	2.26 ± 0.06[a]	1.97 ± 0.03[b]	0.39 ± 0.02[c]
Shikimic acid	0.02 ± 0.00	0.02 ± 0.00	tr
Ascorbic acid	nd	nd	tr
Citric acid	6.44 ± 0.85[a]	6.14 ± 0.14[a]	1.55 ± 0.00[b]
Succinic acid	7.00 ± 0.21[a]	5.74 ± 0.13[b]	1.94 ± 0.05[c]
Fumaric acid	0.01 ± 0.00	0.01 ± 0.00	tr
Total (g/100 g dw)	24.42 ± 1.32[a]	23.35 ±0.65[a]	7.30 ± 0.03[b]

In each row different letters mean significant differences ($p<0.05$); tr- traces; nd-not detected; dw- dry weight.

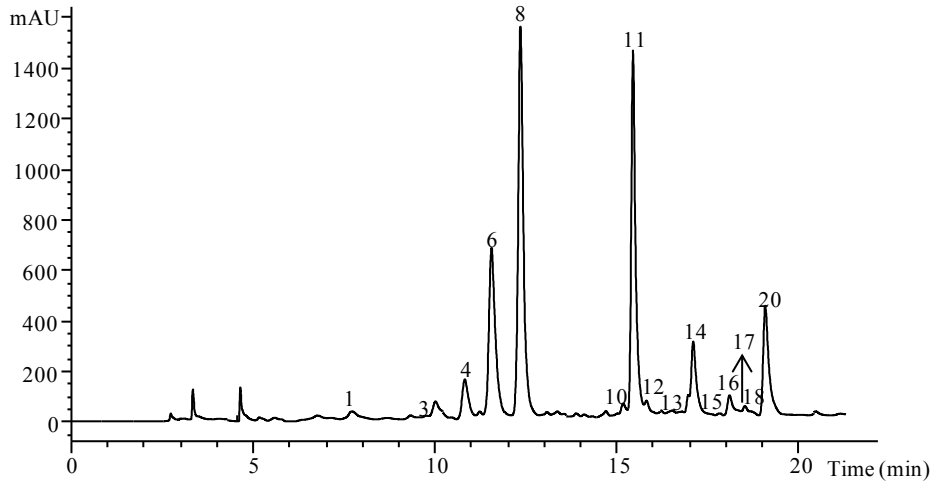
Table 3. Retention time (Rt), wavelengths of maximum absorption in the UV-vis region (λ_{max}), pseudomolecular and MS^2 fragment ions (in brackets, relative abundances) and tentative identification of the phenolic compounds of wild *Matricaria recutita*.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification
1	7.69	328	353	191(100), 179(53), 173(5), 161(5), 135(48)	3-*O*-caffeolyquinic acid
2	8.24	294	153	109(100)	Protocatechuic acid
3	9.75	320	515	515(100),353(5), 191(33), 179(6), 161(13)	1,5-Dicaffeolyquinic acid
4	10.84	326	353	191(75), 179(84), 173(100), 161(11), 135(77)	4-*O*-Caffeolyquinic acid
5	11.34	326	353	191(100), 179(7), 173(3), 161(4), 135(2)	*cis*-5-*O*-Caffeolyquinic acid
6	11.57	326	353	191(100), 179(15), 173(8), 161(15), 135(7)	*trans*-5-*O*-Caffeolyquinic acid
7	12.24	312	355	193(100), 179(13), 149(80)	*cis*-Ferulic acid hexoside
8	12.39	312	355	193(100), 179(13), 149(81)	*trans*-Ferulic acid hexoside
9	14.85	328	367	193(16), 191(100), 173(19), 134(8)	5-*O*-Feruloylquinic acid
10	15.18	354	479	317(100)	Myricetin *O*-hexoside
11	15.45	322	711	549(3), 355(36), 193(100), 149(84)	Ferulic acid hexoside dimer
12	15.81	322	677	515(100), 497(2), 353(17), 335(7), 191(4), 179 (7), 173 (1), 135 (3)	1,3,5-*O* or 1,4,5-*O*-Tricaffeolyquinic
13	16.54	340	651	489(96), 447(55), 285(45)	Luteolin acetylhexoside hexoside
14	17.11	330	515	515 (100),353(66), 335(25),299(2),255(3),203(3),191(26),179(53),173(68), 161(9)	3,4-*O*-Dicaffeolyquinic acid
15	17.68	340	447	285(100)	Luteolin-7-*O*-glucoside
16	18.09	330	515	353(100),335(8),191(89),179(75),173(11),161(7), 155(2),135(28)	3,5-*O*-Dicaffeolyquinic acid
17	18.54	372	505	343(8), 301(100)	Quercetin 7-*O*-acetylhexoside
18	18.64	343	489	327(5), 285(64)	Luteolin *O*-acylhexoside
19	18.81	328	515	353(100),335(4),299(5),255(5),203(3),191(30),179(67), 173(95), 161(2),155(3),135(24)	4,5-*O*-Dicaffeoylquinic acid
20	19.10	296(sh),336	489	327(5), 285(64)	Luteolin *O*-acylhexoside

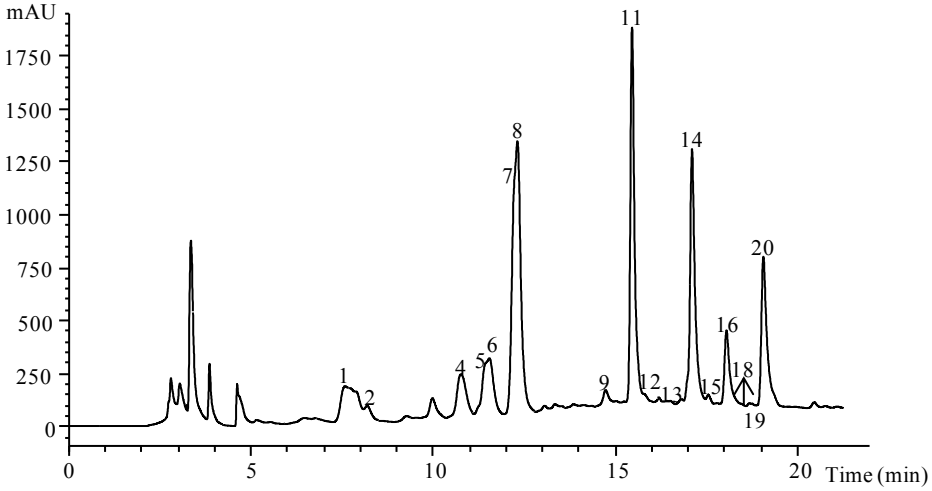
Table 4. Quantification of phenolic compounds in wild *Matricaria recutita* (mean ± SD).

Peak	Infusion	Decoction	Plant (control)
3-*O*-caffeolyquinic acid	0.15 ± 0.01[a]	0.12 ± 0.00[b]	0.07 ± 0.00[c]
Protocatechuic acid	0.07 ± 0.01[a]	0.05 ± 0.01[b]	nd
1,5-Dicaffeolyquinic acid	nd	tr	0.02 ± 0.00
4-*O*-Caffeolyquinic acid	0.21 ± 0.00[b]	0.13 ± 0.00[c]	0.24 ± 0.00[a]
cis 5-*O*-Caffeolyquinic acid	0.17 ± 0.04[a]	0.15 ± 0.00[a]	nd
trans 5-*O*-Caffeolyquinic acid	0.26 ± 0.04[b]	0.22 ± 0.01[b]	1.02 ± 0.02[a]
cis Feruloyl hexoside acid	0.32 ± 0.02[a]	0.28 ± 0.01[b]	nd
trans Feruloyl hexoside acid	0.46 ± 0.01[b]	0.38 ± 0.01[c]	1.02 ± 0.00[a]
5-*O*-Feruloylquinic acid	0.03 ± 0.00[a]	0.02 ± 0.00[b]	nd
Myricetin *O*-hexoside	nd	nd	0.05 ± 0.00
Feruloyl hexoside acid dimer	0.59 ± 0.00[b]	0.55 ± 0.01[c]	0.91 ± 0.00[a]
1,3,5-*O* or 1,4,5-Tricaffeolyquinic	0.03 ± 0.00[b]	0.02 ± 0.00[c]	0.06 ± 0.00[a]
Luteolin acetylhexoside hexoside	0.02 ± 0.00[c]	0.03 ± 0.00[b]	0.11 ± 0.01[a]
3,4-*O*-Dicaffeolyquinic acid	0.73 ± 0.03[a]	0.33 ± 0.01[b]	0.35 ± 0.00[b]
Luteolin-7-*O*-glucoside	0.17 ± 0.01[a]	0.09 ± 0.00[b]	0.06 ± 0.00[c]
3,5-*O*-Dicaffeolyquinic acid	0.26 ± 0.07[a]	0.16 ± 0.00[b]	0.10 ± 0.00[b]
Quercetin 7-*O*-acetylhexoside	nd	nd	0.10 ± 0.00
Luteolin *O*-acylhexoside	0.09 ± 0.01[b]	0.06 ± 0.00[c]	0.19 ± 0.01[a]
4,5-*O*-Dicaffeoylquinic acid	0.17 ± 0.03[a]	0.13 ± 0.01[b]	nd
Luteolin *O*-acylhexoside	1.29 ± 0.12[b]	0.81 ± 0.04[c]	2.10 ± 0.00[a]
Total phenolic acids (g/100 g dw)	3.43 ± 0.22[b]	2.53 ± 0.02[c]	3.99 ± 0.02[a]
Total flavonoids (g/100 g dw)	1.56 ± 0.12[b]	0.98 ± 0.04[c]	2.59 ± 0.01[a]
Total phenolic compounds (g/100 g dw)	5.00 ± 0.33[b]	3.51 ± 0.06[c]	6.58 ± 0.03[a]

In each row different letters mean significant differences ($p<0.05$); tr- traces; nd-not detected; dw- dry weight.



(A)



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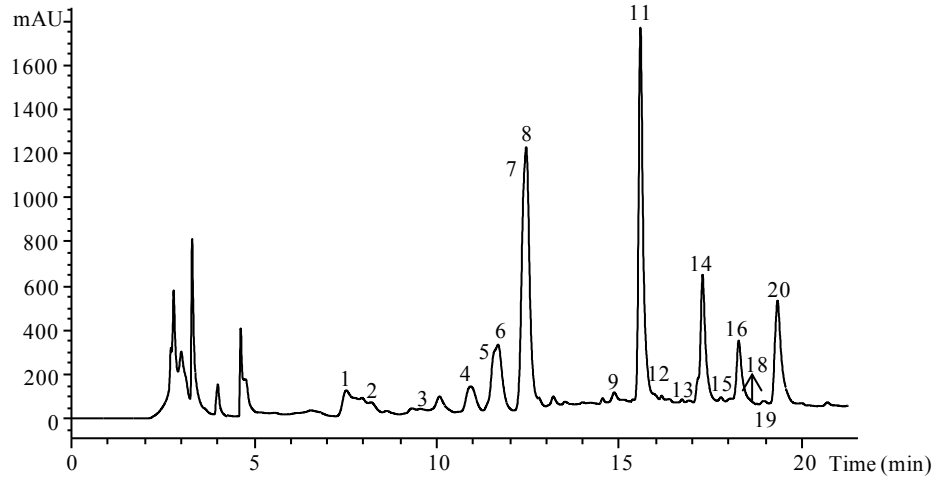

(C)

Figure 1. HPLC chromatogram of the phenolic compounds of *Matricaria recutita* recorded at 280 nm (A) plant methanol extract (control; 1:2 *v/v*); (B) infusion and (C) decoction.