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# 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 recognised potential in drug discovery. Herein, the 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. The decoction exhibited 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 to 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 contained the highest amounts of both 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 pharmaceutical, food and cosmetics industries.


## 1. Introduction

German chamomile (*Matricaria recutita* L.) is an annual herbaceous flowering plant native to Europe. This herb has been used as a herbal remedy for thousands of years (Crevin & Philpott, 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 as a healing medicine (Maschi, Dal Cero, Galli, & Dell' Agli, 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 a variety of 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, Zatloukalová, Filipský, & Hrdina, 2010) and their effects on human health are very often

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ascribed to their potential ability to act by diminishing free radical steady-state concentration in biological systems and so 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 the literature, including reports on antioxidant properties of its methanol extract (Barros, Oliveira, Carvalho, & Ferreira, 2010; Miliauskas, Venskutonis, & van Beek, 2004), antitumour potential of aqueous and organic extracts (Srivastava & Gupta, 2007, 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 characterisation of wild *M. recutita* infusions and decoction.

## 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). Foetal 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. Key morphological 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 an homogenous sample.

### 2.3. Infusion, decoction and methanol extract preparation

To prepare the infusion, 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 infusion obtained was frozen and lyophilized.

To prepare the decoction, 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 decoction obtained 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 °C 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 °C at 150 rpm) for 1 h. The combined methanolic extracts were evaporated at 40 °C (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 for antioxidant activity evaluation by *in vitro* assays. DPPH radical-scavenging activity was evaluated 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 by 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 2 h of assay/initial absorbance) × 100 (Pereira et al., 2012). Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by measuring the decrease 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: $[(A - B)/A] \times 100\%$, 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 a 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 *in vitro* antitumour activity 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 °C, in a humidified air incubator containing 5% $CO_2$. Each cell line was plated at an appropriate density ($7.5 \times 10^3$ cells/well for MCF-7, NCI-H460 and HCT-15 or $1.0 \times 10^4$ 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 °C. 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