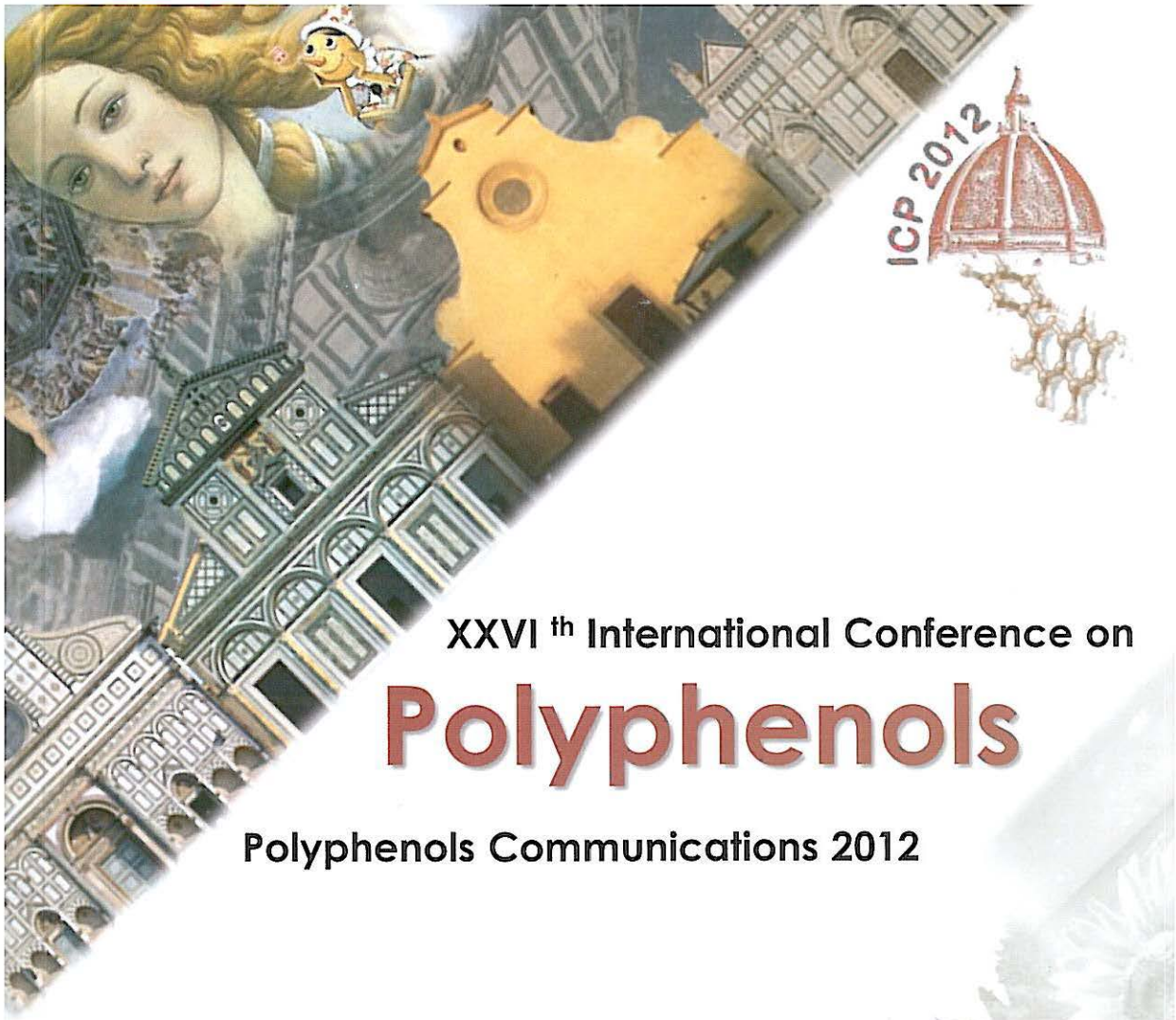

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Comparative study of the phenolic profile and antioxidant properties of *Chamaemelum nobile:* infusion, decoction, and hydroalcoholic extract

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Abstract
This study aimed to determine the phenolic composition and to evaluate the antioxidant activity of different preparations of Roman chamomile: infusion, decoction and methanol:water (80:20) extract. The hydroalcoholic extract revealed the highest antioxidant activity in almost all the performed assays ($EC_{50} \leq 0.62$ mg/mL, depending on the assay), which was in agreement with its highest total content in phenolic compounds. Otherwise, decoctions presented the lowest antioxidant potential ($EC_{50} \leq 1.48$ mg/mL, depending on the assay). The phenolic profile of the different preparations was identical, varying only in the concentrations found. Phenolic acids (caffeoylquinic acids), flavonols (quercetin and kaempferol derivatives) and flavones (apigenin and luteolin derivatives) were found in the three preparations. The most abundant compounds in the infusion and hydroalcoholic extract were 5-*O*-caffeoylquinic acid and an apigenin derivative. These, as also the other phenolic compounds, decreased significantly in the decoction.

Introduction

Roman chamomile, *Chamaemelum nobile* L. (Asteraceae), has been used for centuries as anti-inflammatory, antioxidant, mild astringent, mild sedative, antispasmodic, antibacterial and healing medicine [1]. Oral dosage forms (decoctions and infusions) are used for the symptomatic treatment of gastrointestinal disorders and of the painful component of functional digestive symptoms. External applications of extracts and lotions are recommended as repellent, emollient, in the treatment of skin disorders and for eye irritation or discomfort of various etiologies. Furthermore, it is used as an analgesic in diseases of the oral cavity, oropharynx or both and as a mouthwash for oral hygiene [2]. Different classes of bioactive constituents are present in chamomile, including phenolic compounds. This study aimed to determine the phenolic composition and to evaluate the antioxidant activity of different types of preparations of wild chamomile: infusion, decoction and methanol:water (80:20) extract.

Materials and Methods

Samples and samples preparation. Plant material (leafy inflorescences) was gathered in June 2011 in pastures and grassy roadsides from different individuals randomly selected, according local consumers' criteria. Samples included flower heads, bracts and some leaves and were freeze-dried and kept in the best conditions (-20°C, ~30 days) for subsequent use. For hydroalcoholic extract preparation, the sample (1 g) was extracted twice with methanol:water (80:20; 30 mL each) for 1h. After filtration and evaporation of the methanol (35°C), the extracts were lyophilised. For decoction preparation, the sample (1 g) was added to 200 mL of distilled water, heated 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. For infusion preparation, the sample (1 g) 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.

Phenolic composition. The analysis of phenolic compounds was carried out by reversed-phase HPLC-DAD-ESI/MS [3], in order to establish the phenolic profile of each preparation.
Antioxidant activity. The antioxidant activity was assessed by four *in vitro* assays: scavenging effects on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, reducing power, inhibition of β-carotene bleaching and inhibition of lipid peroxidation in brain cell homogenates by TBARS (thiobarbituric acid reactive substances) assay [4]. Trolox was used as standard.

Results and Discussion
The hydroalcoholic extract revealed the highest antioxidant activity in almost all the assays and the highest content in phenolic compounds (Table 1). Otherwise, decoctions presented the lowest antioxidant potential.

Table 1. Antioxidant activity (EC_{50} values, mg/mL) and quantification of phenolic compounds (mg/g) in different *Chamaemelum nobile* preparations.

	Hydroalcoholic extract	Decoction	Infusion
DPPH scavenging activity	0.62 ± 0.01^{b}	1.48 ± 0.07^{a}	0.41 ± 0.01^{c}
Reducing power	0.29 ± 0.01^{b}	0.53 ± 0.01^{a}	0.26 ± 0.01^{b}
β-carotene bleaching inhibition	0.44 ± 0.00^{c}	0.68 ± 0.07^{b}	1.22 ± 0.03^{a}
TBARS inhibition	0.08 ± 0.01^{c}	0.27 ± 0.01^{a}	0.12 ± 0.00^{b}
Total flavonols derivatives	3.38 ± 0.27^{a}	0.21 ± 0.01^{c}	1.31 ± 0.12^{b}
Total flavones derivatives	34.80 ± 1.84^{a}	15.09 ± 0.15^{c}	21.78 ± 0.26^{b}
Total caffeoylquinic acids	25.43 ± 1.33^{a}	0.44 ± 0.07^{c}	19.45 ± 0.67^{b}
Total phenolic acid derivatives	1.54 ± 0.19^{b}	0.81 ± 0.11^{c}	1.85 ± 0.03^{a}
Total phenolic compounds	65.15 ± 3.62^{a}	16.55 ± 0.32^{c}	44.39 ± 1.07^{b}

In each row different letters mean significant differences ($p<0.05$).

The phenolic profile of the different preparations was identical, varying only in the concentrations found. Up to thirty different phenolic compounds were detected in the three preparations, including phenolic acids (hydroxycinnamic derivatives mainly, caffeoylquinic acids), flavonols (quercetin, kaempferol and myricetin derivatives) and flavones (apigenin and luteolin derivatives), whose identities were established based on their chromatographic characteristics and absorption spectra, as compared with our data library, molecular ions and MS^2 fragmentation patterns.
The most abundant compounds in infusion and hydroalcoholic extract were 5-*O*-caffeoylquinic acid and an apigenin derivative. Degradation was found in both infusion and decoction preparation (Table 1), mainly in the last one. This could be related to the higher thermical impact applied to obtain this type of preparation, when compared to the infusion. Overall, the present study proved the lost of antioxidant potential (antioxidant properties and phenolic compounds) in chamomile decoction, revealing the hydroalcoholic extract the highest antioxidant activity and highest amounts of phenolic compounds.

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