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Phenolic composition and antioxidant activity of Portuguese wild edible greens

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Abstract. This study aimed to determine the phenolic composition and to evaluate the antioxidant activity of wild asparagus, white and black bryony (early shoots in spring). Black bryony revealed the highest antioxidant potential in all the assays (EC_{50}<203 μg/ml), which is compatible to its highest concentration in phenolic compounds. The main phenolic compounds found in asparagus and black bryony were glycoside derivatives of flavonols (kaempferol, quercetin and isorhamnetin, the latter only found in asparagus) and phenolic acids (sinapic and chlorogenic acid, respectively for asparagus and black bryony). Black bryony revealed the highest content of these compounds. White bryony sample can be differentiated from the other two wild edible greens presenting flavones as main compounds being identified glycoside derivatives of luteolin and apigenin.

Introduction. Mediterranean diet used to include various wild greens, which were traditionally collected and consumed in different manners. *Asparagus acutifolius* (wild asparagus), *Bryonia dioica* (white bryony) and *Tamus communis* (black bryony) are same examples of those edible wild greens. The most used species are wild asparagus and white bryony, whilst black bryony is less known and only eaten locally in the northeastern region. The edible portions of these species are the young shoots, without flower buds, available during a short period, especially in the case of both bryonies, because traditional knowledge warns against their toxicity and considers the young shoots and tendrils the least toxic part. Fruits, vegetables and beverages contain a significant amount of flavonoids (flavonols, flavones, flavanones, flavans and anthocyanins). While there is no direct evidence that these antioxidants are central to the benefits of the Mediterranean Diet, indirect evidence from epidemiological data and the increasing understanding of their mechanisms of action suggest that antioxidants may play a major role [1]. The present work aims to describe the phenolic profile and antioxidant activity of these wild edible greens.

Materials and Methods

Extraction. Samples (1 g) were 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.

Total phenolics. They were determined by the *Folin-Ciocalteu* colorimetric assay and total flavonoids were determined spectrophotometrically using the method based on the formation of a complex flavonoid-aluminum [2]. Results were expressed as gallic acid equivalents (GAE) and catechin equivalents (CE), respectively.

Phenolic composition. The extracts were analyzed by HPLC-DAD-ESI/MS as described elsewhere [3].
Antioxidant activity. It was accessed by four *in vitro* chemical and biochemical assays: scavenging effects on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, Fe(III) reducing power, inhibition of β-carotene bleaching and inhibition of lipid peroxidation in brain cells homogenates by TBARS (thiobarbituric acid reactive substances) assay [2]. Results were expressed as EC_{50} values and Trolox was used as standard.

Results and Discussion. Results obtained in the analysis of the different samples are shown in **Table 1.** High amounts of phenolics were found in the samples analysed. Black bryony was the wild green that presented the highest content in phenolic compounds. All the samples showed relevant antioxidant activity (EC_{50} values lower than 0.5 mg/ml) in the order of black bryony > asparagus > white bryony.

Table 1. Extraction yields, composition in phenolics and flavonoids, and antioxidant activity (EC_{50} values, μg/ml) of the edible wild greens (mean ± SD; n=3).

	Asparagus	White bryony	Black bryony
η (%)	29.73 ± 1.56	48.24 ± 3.28	23.37 ± 2.51
Phenolics (mg GAE/g extract)	623.73 ± 27.68	258.24 ± 21.95	758.99 ± 28.96
Flavonoids (mg CE/g extract)	57.83 ± 2.40	18.09 ± 1.18	149.83 ± 11.63
DPPH scavenging activity	422.77 ± 24.14	639.51 ± 49.25	202.69 ± 30.20
Reducing power	191.43 ± 12.42	204.13 ± 9.06	68.07 ± 3.56
β-carotene bleaching inhibition	165.62 ± 6.95	370.54 ± 4.69	70.70 ± 5.29
TBARS inhibition	104.82 ± 3.93	196.81 ± 9.71	94.67 ± 6.40

Significantly negative linear correlations were established between the phenolics and flavonoids contents, and EC_{50} values of the four methods used, proving that the wild green with the highest bioactive compounds content is the most efficient in antioxidant activity. The correlations were slightly more significant for flavonoids than for phenolics, and the highest determination coefficients were obtained for DPPH and β-carotene bleaching inhibition assays.

Samples of wild asparagus and black bryony presented glucosides of flavonols as main phenolics, while white bryony presented glucosides of flavones. Among seven flavonols found, quercetin derivatives were the main compounds (30.11 mg/Kg of extract) in black bryony. In Asparagus sample, quercetin-3-O-rutinoside was the majority flavonol (6.08 mg/Kg of extract); this compound has been reported to play a significant role in the antioxidant activity of this vegetable [4]. In white bryony four flavones were found, being apigenin 6-C-glucoside-7-O-glucoside (11.71 mg/Kg of extract) the main one.

Black bryony, wild asparagus and white bryony shoots proved to be vegetables with high antioxidant activity and important antioxidant compounds, including flavonoids. Antioxidants can scavenge free radicals and protect the human body from oxidative stress, which is in the origin of some pathologies, like cancers and heart diseases, and physiological processes like ageing.

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