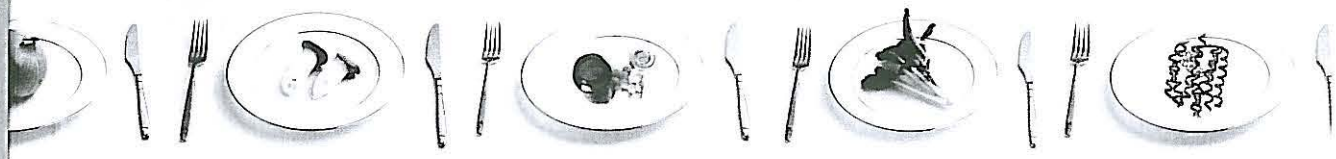

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Evaluation of the *in vitro* antioxidant activity of three Lamiaceae often used in Portuguese folk medicine

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KEYWORDS
Lamiaceae, Antioxidants, Radical scavenging capacity, Lipid peroxidation inhibition

SUMMARY
This study reports the first approach to the antioxidant potential evaluation of three Lamiaceae often used in Portuguese folk medicine. The lipid peroxidation inhibition capacity of ground ivy, oregano and mastic thyme was accessed by biochemical assays used as models for the lipid peroxidation damage in biomembranes, namely inhibition of β-carotene bleaching in the presence of linoleic acid radicals and inhibition of thiobarbituric acid reactive substances (TBARS) formation in brain homogenates. The antioxidant properties were also evaluated through the reducing power determination and radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Bioactive compounds such as phenolics, flavonoids and ascorbic acid were also determined. Oregano proved to have the highest radical scavenging and lipid peroxidation inhibition capacity (EC_{50} values lower than 0.45 mg/ml). This species also revealed the highest content in antioxidants such as phenolics (368.58 ± 18.18 mg/g), flavonoids (224.15 ± 0.96 mg/g) and ascorbic acid (170.69 ± 5.29 μg/g). Significantly negative linear correlations were observed between the bioactive compounds and antioxidant activity EC_{50} values of the three Lamiaceae.

INTRODUCTION
In recent years oxidative stress, induced by reactive oxygen species (ROS) that are generated by normal metabolic activity as well as lifestyle factors such as smoking, exercise and diet, have been implicated in the causation and progression of several chronic diseases. Antioxidants that can mitigate the damaging effects of ROS have been the focus of recent research (Hallliwell, 1996). Epidemiological studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk of certain forms of cancer and cardiovascular diseases (Prior, 2003). Many wild plants gathered from the scrubland were preserved and used for medicinal and food purposes in the north-eastern region of Portugal (Santayana et al., 2007). Since they are important ingredients of the folk pharmacopoeia and traditional cuisine some of these wild botanicals have been semi-domesticated and are still cultivated in homegardens and present in every homesteads. Several ethnobotanical surveys conducted in this Portuguese region have highlighted the use of three particular Lamiaceae. Ground ivy (*Glechoma hederaceae*), oregano (*Origanum vulgare* subsp. *virens*) and mastic thyme (*Thymus mastichina*) are widespread Mediterranean perennial herbs widely considered as medicinal plants, although it has also been reported some other common uses. Epidemiological and experimental studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk for chronic diseases, such as

cardiovascular diseases, arthritis, chronic inflammation and cancers. These physiological functions may be partly attributed to the abundance of antioxidants such as vitamin C, vitamin E, β-carotene and phenolics.

MATERIALS AND METHODS

Samples and sample's preparation. Samples of leaves and steams of *Glechoma hederaceae* (ground ivy) and inflorescences of *Origanum vulgare* subsp. *virens* (oregano) and *Thymus mastichina* (thyme) were gathered in Bragança, Trás-os-Montes, North-eastern Portugal. A fine dried powder was extracted by stirring with methanol at 25 ºC at 150 rpm for 12 h and filtered through Whatman No. 4 paper. The residue was then extracted with one additional portion of methanol. The combined methanolic extracts were evaporated to dryness.

Determination of antioxidants. Total phenolics were estimated by the *Folin-Ciocalteu* colorimetric assay (Wolfe et al., 2003). Gallic acid was used to calculate the standard curve, and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract. Total flavonoids were determined spectrophotometrically using the method based on the formation of a complex flavonoid-aluminum (Barros et al., 2008). (+)-Catechin was used to calculate the standard curve and the results were expressed as mg of (+)-chatequin equivalents (CEs) per g of extract. Ascorbic acid was determined according to the 2,6-dichloroindophenol assay (Barros et al., 2008), on the basis of the calibration curve of authentic L-ascorbic acid; the results were expressed as mg of ascorbic acid/g of dry weight.

Evaluation of antioxidant activity

DPPH radical-scavenging activity. This methodology was performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc). The reaction mixture in each one of the 96-wells consisted of extract solution and aqueous methanolic solution containing DPPH radicals. The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm.

Reducing power. This methodology was performed using the Microplate Reader described above. The presence of reducers (i.e. antioxidants) causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm we can monitor the Fe^{2+} concentration; a higher absorbance at 700 nm indicates a higher reducing power.

Inhibition of β-carotene bleaching. The antioxidant activity of the extracts was evaluated by the β-carotene linoleate model system, as described previously by us (Barros et al., 2008) and measured at 470 nm. The linoleic acid free radical attacks the highly unsaturated □-carotene models. The presence of different antioxidants can hinder the extent of □-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS). It was measured the colour intensity of the malondialdehyde (MDA)-TBA complex formed in the system by its absorbance at 532 nm (Barros et al., 2008).

RESULTS AND DISCUSSION

Table 1 presents the bioactive compounds and antioxidant activity EC_{50} values of the three Lamiaceae. Phenolics, and particularly flavonoids, were the main antioxidant compounds found in the samples. *Origanum vulgare* gave the best results in all the antioxidant activity assays (EC_{50}

values ≤ 0.45 mg/ml), which is in agreement with the highest content in phenolics (368.58 ± 18.18 mg GAE/g) and flavonoids (224.15 ± 0.96 mg CE/g) found in this species. The very low EC_{50} value (0.01 mg/ml) obtained for TBARS inhibition in brain homogenates is very promising, considering that brain is highly sensitive to oxidative damage.

Table 1. Bioactive compounds and antioxidant activity EC_{50} values of *Glechoma hederacea*, *Thymus mastichina* and *Origanum vulgare* obtained using the best extraction conditions. The results are expressed as mean ± SD (n=3). In each row different letters mean significant differences ($p<0.05$).

		G. hederacea	*O. vulgare*	*T. mastichina*
Bioactive compounds	Phenolics (mg GAE/g)	196.61± 6.09b	368.58 ± 18.18a	165.29 ± 1.11c
	Flavonoids (mg CE/g)	95.02 ± 2.73b	224.15 ± 0.96a	83.85 ± 1.42c
	Ascorbic acid (mg/g dry weight)	0.17 ± 0.00a	0.17 ± 0.01a	0.13 ± 0.00b
Antioxidant activity	DPPH scavenging Activity	0.39 ± 0.02b	0.16 ± 0.03c	0.69 ± 0.04a
	Reducing Power	0.22 ± 0.00b	0.18 ± 0.00c	0.23 ± 0.00a
	β-carotene bleaching inhibition	0.87 ± 0.10a	0.45 ± 0.05b	0.90 ± 0.09a
	TBARS Inhibition	0.11 ± 0.01b	0.01 ± 0.00c	0.43 ± 0.02a

The same subspecies of *Origanum vulgare* was studied in Spain and the EC_{50} values obtained for methanolic extracts were 14.14 ± 0.32 µg/ml for inflorescences and 18.17 ± 1.38 µg/ml for steam and leaves (López et al., 2007). Our results were more similar to the ones obtained by the same authors with another subspecies, *Origanum vulgare* subs. *vulgare* (inflorescences 185.58 ± 12.39 µg/ml; steam and leaves 57.01 ± 7.30 µg/ml).

Significantly negative linear correlations (Figure 1) were established between the phenolics and flavonoids content, and EC_{50} values of DPPH scavenging activity (determination coefficient 0.7931 for phenolics and 0.7454 for flavonoids; $p < 0.001$), reducing power (determination coefficient 0.9647 for phenolics and 0.9515 for flavonoids; $p < 0.001$), β-carotene bleaching inhibition (determination coefficient 0.873 for phenolics and 0.8468 for flavonoids, $p<0.001$) and TBARS inhibition (determination coefficient 0.6037 for phenolics and 0.5392 for flavonoids, $p<0.001$). This proves that the extraction methodology was adequate, being the sample with the highest bioactive compounds content the most efficient in antioxidant activity (with the lowest EC_{50} values).

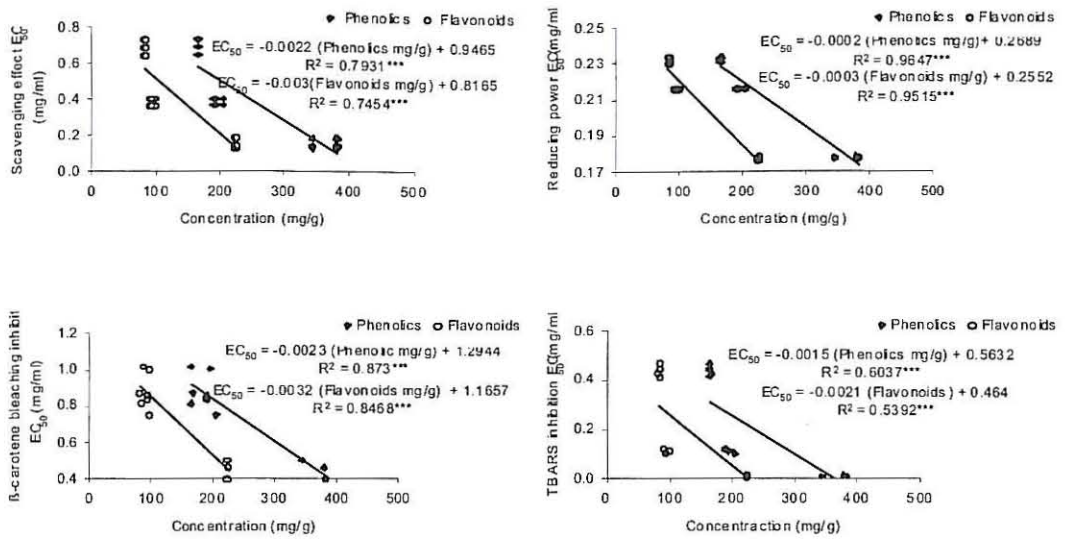

Fig. 1. Correlations established between phenolics and flavonoids contents and EC_{50} values obtained in the antioxidant activity assays.

CONCLUSIONS

Overall, it is noteworthy to point out that the scientific literature contains no studies that validate the possible beneficial effects of the Portuguese medicinal herbs, particularly related to their antioxidant activity. Therefore, the report of the radical scavenging activity and lipid peroxidation inhibition capacity of these Lamiaceae from North-eastern Portugal could help in the explanation of their uses in folk medicine against several chronic diseases known to be related to the production of ROS and oxidative stress.

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