Antioxidant properties and phytochemical analysis of an uncharacterized Portuguese endemic aromatic herb, *Thymus capitellatus*

Dina M. Carrilho[1]*, Lucélia R. Tavares[1], David Barata[1], Cláudia Santos[1], Teresa Serra[2], Rui Duarte[3], Catarina Duarte[2], Dalila Espírito-Santo[4], Artur Teixeira[5], Ricardo Ferreira[1,5]. [1]Disease and Stress Biology Laboratory, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal; [2] Nutraceuticals and Delivery Laboratory, Instituto de Biologia Experimental e Tecnológica, Apartado 127, 2781-901 Oeiras, Portugal; [3] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; [4] Herbário João de Carvalho e Vasconcellos, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal; [5] Departamento de Botânica e Engenharia Biológica, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal; *carrilho@itqb.unl.pt

Abstract. Deterioration of the nutritional value, organoleptic properties and safety of food is mainly caused by oxidation. Therefore, antioxidants are being used as preservatives in foods. Due to consumer's concerns about safety of synthetic antioxidants, there is an increasing interest in the use of natural compounds. More recently, spices and aromatic herbs have received increased attention as sources of many effective antioxidants such as polyphenols.

Among the aromatic plants, *Thymus* species, including the well-known *Thymus vulgaris* L., are sources of natural antioxidants whose properties have been extensively studied. Thyme has been used in the Mediterranean area since ancient times for its medicinal, aromatic and also culinary properties.

In the present work, the polyphenol and flavonoid contents, as well as the antioxidant activity of *Thymus capitellatus* leaves (which is an endemic thyme in the Southwest of Portugal) were investigated. Three different leaf extractions were performed using exclusively clean solvents.

The hydroethanolic extract presented the highest polyphenol content (12.8±0.2 mg GAE.g^{-1} dw), followed by the ethanolic and water extracts. However, the flavonoid content of the ethanolic extract (8.7±0.7 mg catechin equivalents.g^{-1} dw) was higher than those of the other two extracts.

Two assays were used to compare the antioxidant ability: peroxyl radical scavenging capacity by ORAC and hydroxyl radical scavenging by EPR. Both hydroethanolic and ethanolic extracts from *T. capitellatus* exhibited strong antioxidant activities (449±57 and 384±25 µmol TE.g^{-1} dw, respectively). These results were confirmed by EPR and are similar to those reported for *T. vulgaris* extracts.

Therefore, the use of this endemic spice as source of antioxidant compounds is a promising alternative to the use of synthetic antioxidants in food preservation. Besides, adding fresh thyme to salads will not only enhance the flavour but will help to preserve the uncooked food and contribute significantly to the total intake of natural antioxidants.

Introduction. The interest in polyphenols, including flavonoids, as preservatives in foods has considerably increased mainly due to their antioxidant effects. Spices and herbs constitute natural sources of antioxidants being a promising alternative to the synthetic antioxidants. Among the aromatic plants belonging to the family Lamiaceae, the genus *Thymus* is noteworthy for the numerous species and varieties of wild-growing plants, many typical for the Mediterranean area. The well-known species *Thymus vulgaris* L., which has been extensively studied, is commonly used as a culinary herb spice for adding flavour but also for medicinal purposes.

In the present work, the polyphenol and flavonoid contents, as well as the antioxidant activity of *Thymus capitellatus* leaves (endemic from Southwest of Portugal) were investigated.

Materials and Methods. Three extractions from leaves were performed with clean solvents (6 mL.g^{-1} fw): water; water/ethanol mixture 1:1 and ethanol. Determination of total phenolic compounds was performed by the Folin-Ciocalteau method [1] and gallic acid (GA) was used as standard. Determination of flavonoid content was performed by a modification of $AlCl_3$ complexation method [2], using (+)-catechin hydrate as standard. Peroxyl radical scavenging capacity was determined by ORAC (Oxygen Radical Absorbance Capacity) method [3,4] and hydroxyl radical scavenging was measured by EPR (Electron Paramagnetic Resonance) [5].

Results and Discussion. In order to evaluate the polyphenol and flavonoid contents and, therefore, the antioxidant activities of *T. capitellatus* leaves, three different extractions with clean solvents were tested to choose the best solvent to extract the highest antioxidant activity.

The hydroethanolic extract showed the highest polyphenol content (12.8±0.2 mg GA Eq.g^{-1} dw), followed by the water and ethanolic extracts (Fig.1). However, the flavonoid content of the ethanolic extract (8.7±0.7 mg (+)catechin Eq.g^{-1} dw) was higher than those of the other two extracts (Fig.1).

Two assays were used to compare the antioxidant ability: peroxyl radical scavenging capacity by ORAC and hydroxyl radical scavenging by EPR.

As determined by ORAC, the hydroethanolic and ethanolic extracts from *T. capitellatus* showed the highest capacity to scavenge peroxyl radicals (449±57 and 384±25 µmol Trolox Eq.g^{-1} dw, respectively) (Fig.2), thus indicating strong antioxidant activities which are similar to those reported for *T. vulgaris* extracts.

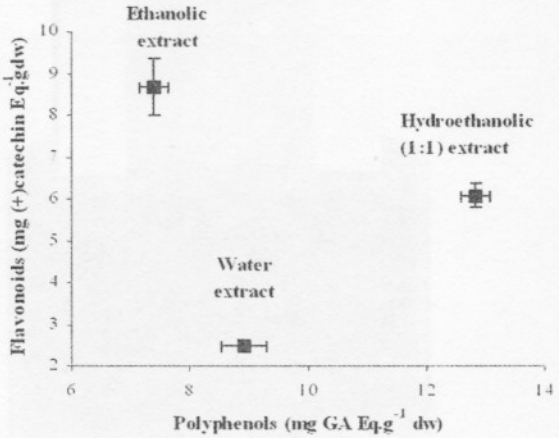

Fig. 1. *Polyphenol and flavonoid content of T. capitellatus leaf extracts.*

EPR analysis of the hydroethanolic extract produced a signal decrease of 64.2 %, demonstrating the ability of this extract to scavenge hydroxyl radicals (inset in Fig.2).

In conclusion, the hydroethanolic *T. capitellatus* leaf extract was found to be an effective scavenger of both peroxyl and hydroxyl radicals, thus confirming its strong antioxidant activity. Moreover, these results indicate that the water:ethanol mixture (1:1) is the best solvent to extract the antioxidant activity.

Therefore, the use of this endemic herb as source of antioxidant compounds is a promising alternative to the use of synthetic antioxidants in food preservation. Besides, adding fresh thyme to salads will not only enhance the flavour but will help to preserve the uncooked food and contribute significantly to the total intake of natural antioxidants.

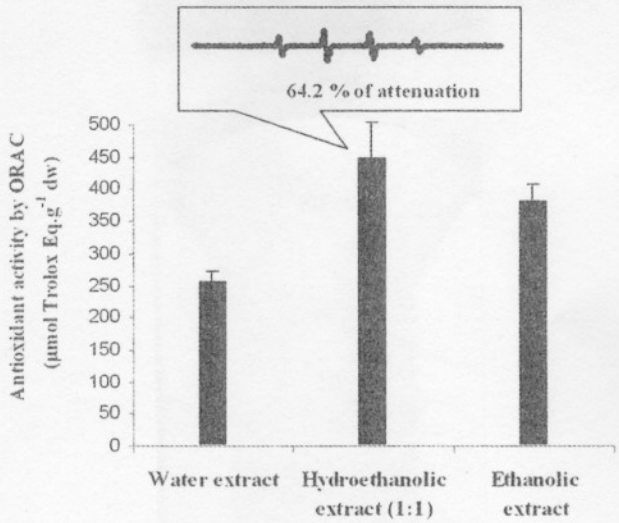

Fig. 2. *Antioxidant activities by ORAC of T. capitellatus leaf extracts. Inset: Antioxidant activity by EPR of T. capitellatus leaf hydroethanolic extract.*

References

[1] Singleton VL, Rossi JA (1965) *Am. J. Enol. Vitic.*16: 144-158.
[2] Michalska A, Ceglinska A, Zielinski H (2007) *Eur. Food Res. Technol.*225: 545-551.
[3] Cao G, Alessio HM, Cutler RG (1993) *Free Radic. Biol. Med.*14: 303-311.
[4] Wang SY, Lin HS (2000) *J. Agric. Food Chem.*48: 140-146.
[5] Leonard SS, Xia C, Jiang BH, Stinefelt B, Klandorf H, Harris GK, Shi X (2003) *Biochem. Biophys. Res. Commun.*309: 1017-1026.