



PHENOLIC CHARACTERIZATION AND ANTIOXIDANT ACTIVITY

INTRODUCTION



Thymus citriodorus, also known as Lemon thyme, is a *Lamiaceae* subshrub aromatic and medicinal plant cultivated in the Mediterranean region. It is frequently used as a deodorant, antiseptic and antifungal element, as well as in the treatment of asthma and other respiratory diseases [1]. As part of beneficial activities of this are due to the volatile constituents, its essential oil composition has been the focus of many investigations. In contrast, there is only a limited number of data on the composition of other bioactive phytochemicals of *Thymus*, such as their phenolic compounds.

METHODS

The phenolic compounds of *Thymus citriodorus* were obtained by extraction with aqueous ethanol (80%). The total phenolic compounds of the ethanolic extract (Fig 1) were determined by an adaptation of the Folin-Ciocalteu procedure [2] and the flavones/ flavonols content was accessed following the procedure of Popova *et al* [3]. The phenolic characterization was performed by fractionation of the extract by reversed-phase HPLC and further analysis of the major phenolic compounds by ESI-MS and MSⁿ. The HPLC analysis was performed on a RP-C18 column 250 mm× 4 mm id, 5µm bead diameter (Temperature of 30°C, flow rate of 1 mL/min). The mobile phase comprised (A) 0,1% formic acid in water and (B) 0,1% formic acid in acetonitrile and the solvent gradient started with 90% A and 10% B, reaching 40% B at 30 min, 5% B at 40 min, then returning to the initial conditions at 50 min. The antioxidant activity was accessed by measuring the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging potential [4] and its reducing power[5].



Fig 1- Ethanolic extract of *Thymus citriodorus*

RESULTS AND DISCUSSION

Table 1- Extraction yields, phenolic content and antioxidant capacity of *Thymus citriodorus*

Mass (% of dry weight)	Total Phenolics ^a (mg/g fraction)	Flavones/ Flavonols ^b (mg/g fraction)	DPPH (EC ₅₀) ^c (mg/ml)	Reducing Power (EC ₅₀) ^d (mg/ml)
17.1	138.8±13.6	27.3±2.2	0.32±0.05	0.77±0.15

Values are means ± S.D. of three replicate analyses;

^a Data expressed as milligrams of gallic acid equivalents (GAE) per gram of extract;

^b Data expressed as milligrams of quercetin equivalents(QE) per gram of extract;

^c EC₅₀ – Concentration for a 50% inhibition;

^d EC₅₀ – Effective concentration at which the absorbance was 0.5.

The total amount of phenolic compounds in the ethanolic extract of *Thymus citriodorus* accounted for 138.8±13.6 mg/g and its content of flavones/flavonols was estimated as 27.3±2.2 mg/g. Also, it exhibited a high antioxidative capacity, with EC₅₀ values of 0.32±0.05 mg/ml and 0.77±0.15 mg/ml, for scavenging potential and reducing power, respectively (Table 1).

CONCLUSION

The overall results suggest that the phenolic extract of the *Thymus citriodorus* can be claimed as a good source of natural antioxidants. The extract is mainly composed of glucosides of luteolin, naringenin and eriodictyol and of less common compounds, namely the eriodictyol-7-*O*-glucuronide, eriodictyol-3',7-di-*O*-glucoside, quercetagenin-dimetil-*O*-hexoside, chrysoeriol-7-*O*-glucoside (Fig 2 and Table 2).

REFERENCES

- [1]Horwath, A.B. *et al.* (2008). *Biochem. Syst. Ecol.* 36, 117-33
- [2]Guyot, S. *et al.* (1998). *J. Agric. Food Chem.* 46, 1698-705
- [3]Popova, M. *et al.* (2004). *Phytoch. Anal.* 15, 235-40
- [4]Ferreira, A. *et al.* (2006). *J. Ethnopharmacol.* 108, 31-7
- [5]Pereira, J. *et al.* (2006). *J. Agric. Food Chem.* 54, 8425-8431

Phenolic characterization by fractionation of the *Thymus citriodorus* extract by reversed-phase HPLC and analysis by ESI-MS and MSⁿ.

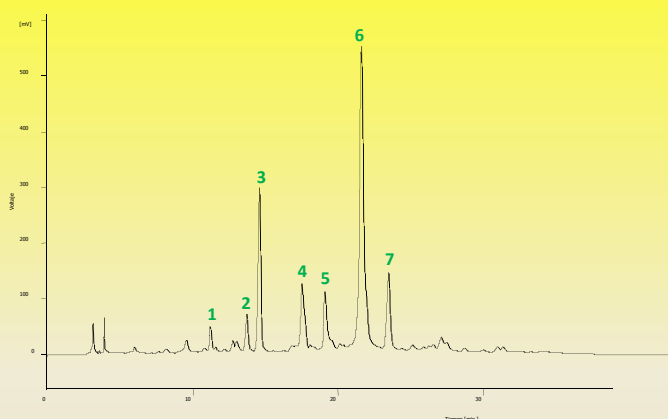


Fig 2- HPLC/UV profile at 280 nm of the phenolic extract of *Thymus citriodorus*

Peak	RT (min)	Compound	Negative ion [M-H]	Main Fragments ESI-MS ⁿ
1	11.24	<i>p</i> - coumaric acid synapoyl	387	207, 163, 109
		Eriodyctiol-3',7-di- <i>O</i> -glucoside	611	449, 287, 151
2	13.65	Eriodyctiol-7- <i>O</i> -glucoside	449	287, 151, 107
		Quercetagenin-dimetil- <i>O</i> -hexoside	507	345, 327, 315
3	14.62	Eriodyctiol-7- <i>O</i> -glucoside (isomer)	449	287, 151, 107
4	17.51	Luteolin-7- <i>O</i> -glucoside	447	285, 241, 199, 151
5	19.13	Eriodyctiol - 7- <i>O</i> -glucuronide	463	287, 151
		Naringenin-7- <i>O</i> -glucoside	433	271, 151
6	21.62	Luteolin-7- <i>O</i> -glucuronide	461	285, 241, 151
		Chrysoeriol-7- <i>O</i> -glucoside	461	299, 284, 256, 227
7	23.51	Rosmarinic acid	359	197, 179, 161
		Litospermic acid	537	493, 359

Table 2- Mass spectral data of the main phenolic constituents isolated from the *Thymus citriodorus*

ACKNOWLEDGEMENT

Olívia Pereira thanks for the PROTEC grant SFRH/PROTEC/49600/2009 (Programa de apoio à formação avançada de docentes do Ensino Superior Politécnico)