THYMUS CITRIODORUS AS A SOURCE OF ANTIOXIDANTS

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INTRODUCTION

Thymus species are well known as medicinal plants because of their biological and pharmacological properties, which include anti-asthmatic, anti-septic, antimicrobial and antioxidant [1]. It is believed that part of these beneficial effects are due to the volatile constituents of *Thymus*, and thus, their 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* and their potential biological effects.

The present study aims to elucidate the phenolic composition of an ethanolic extract of *Thymus citriodorus*, as well as to determine its antioxidant capacity.

METHODS

The ethanolic extract was obtained by solubilisation of the defatted-dried plant with aqueous ethanol (80%) for twenty minutes, in a total number of five extractions.
The total phenolic compounds of the ethanolic extract were determined by an adaptation of the Folin-Ciocalteau procedure [2].
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 MS^n [3].
The HPLC analysis was performed on a RP-C18 column 250 mm× 4 mm id, 5µm bead diameter (Temperature of 30ºC, flow rate of 1 mL/min). Gradient elution was carried out with a mixture of 0.1% (v/v) of formic acid in water and acetonitrile and the chromatographic profiles were recorded at 280 nm.
The antioxidant activity was accessed by measuring the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging potential [4] and its reducing power [5].

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)	DPPH (EC_{50})[b] (mg/mL)	Reducing Power (EC_{50})[c] (mg/mL)
17.1	139±14	0.32±0.05	0.8±0.2

Values are means ± S.D. of three replicate analyses;
[a] Data expressed as milligrams of gallic acid equivalents (GAE) per gram of extract;
[b] EC_{50} – Concentration for a 50% inhibition;
[c] EC_{50}– Effective concentration at which the absorbance was 0.5.

The phenolic compounds account for 13.9% of the ethanolic extract total and this extract exhibited a high antioxidative capacity, with EC_{50} values of 0.32±0.05 mg/ml and 0.8±0.2 mg/ml, for the DPPH scavenging potential and for the reducing power, respectively (Table 1).

The main phenolic components of the ethanolic extract of *Thymus citriodorus* were luteolin-7-*O*-glucoside (12±2 µg/mg extract), rosmarinic acid (10.4±0.6 µg/mg extract) and apigenin- 7-*O*- glucoronide (9±2 µg/mg extract) (Table 2).

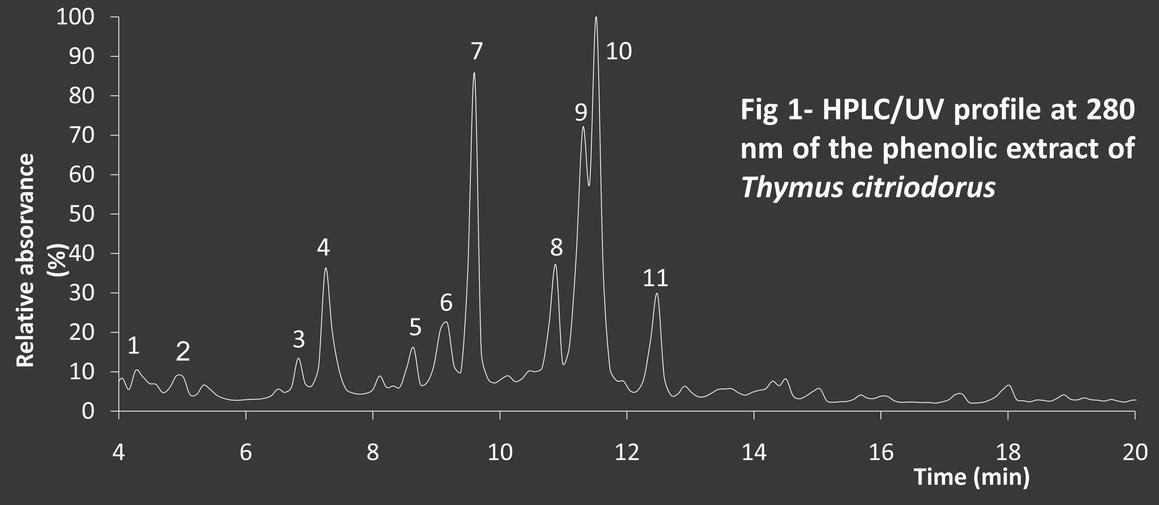

Fig 1- HPLC/UV profile at 280 nm of the phenolic extract of *Thymus citriodorus*

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

Peak	RT (min)	Compound	Standard Compound	Mean content (mg/g extract)
1	4.3	5’ hidroxijasmonic acid 5’-*O*-glucoside Eriodictyol-3’,7-di-*O*-glucoside	E-7-*O*-G	0.71±0.07
2	5.0	Non identified	-	-
3	6.8	Eriodictyol-*O*-glucoside Quercetagetin-dimethyl-ether-*O*-hexoside	E-7-*O*-G	1.3±0.4
4	7.3	Eriodictyol-*O*-glucoside	E-7-*O*-G	3.7±0.5
5	8.6	Luteolin-5-*O*-glucoside	L-7-*O*-G	3.2±0.5
6	9.1	Naringenin-5-*O*-glucoside Eriodictyol- 7-*O*-glucoronil	N-7-*O*-G	1.8±0.2
7	9.6	Luteolin-7-*O*-glucoside Luteolin-7-*O*-glucoronil Sagerinic acid	L-7-*O*-G	12±2
8	10.9	Chrysoeriol-7-*O*-glucoside	-	-
9	11.3	Apigenin- 7-*O*- glucoronide	A-7-*O*-G	9±2
10	11.5	Rosmarinic acid	RA	10.4±0.6
11	12.5	3´-*O*-(8´´-Z-Caffeoyl)rosmarinic acid	RA	2.3±0.9

E-7-*O*-G: Eriodictyol-7-*O*-glucoside; L-7-*O*-G: Luteolin-7-*O*-glucoside; N-7-*O*-G: Naringenin-7-*O*-glucoside; A-7-*O*-G: Apigenin-7-*O*-glucoside; RA: Rosmarinic Acid

CONCLUSION

- **The ethanolic extract of *Thymus citriodorus* has a good antioxidant capacity.**
- **This extract is mostly rich in luteolin-7-*O*-glucoside, rosmarinic acid and apigenin- 7-*O*- glucoronide.**
- **Yet, it also contains phenolic compounds that were not previously found in *Thymus* genus.**
- **New compounds enclose glucosides of common flavonoids and sagerinic acid.**
- **The relevance of the main phenolic component in the beneficial properties of this plant is now under investigation.**

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