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Thymus citriodorus as a source of antioxidants

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Thymus species are well known as medicinal plants because of their biological and pharmacological properties, which include anti-asthmatic, anti-septic, antimicrobial and antioxidant [1,2]. 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. 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 in the extract accounted for 139±14 mg/g, as expressed as gallic acid equivalents. Further analysis of the ethanolic extract by high performance liquid chromatography (HPLC) and electrospray mass spectrometry in the negative mode allowed to conclude that its main phenolic components were rosmarinic acid (14.0±0.8 µg/mg extract), luteolin-7-*O*-glucoside (11±2 µg/mg extract), an apigenin derivative (9±2 µg/mg extract), eriodictyol-*O*-glucoside (5.5±0.7 µg/mg extract) and naringenin-*O*-glucoside (1.6±0.1 µg/mg extract). Moreover, the ethanolic extract of *Thymus citriodorus* exhibited a high antioxidative capacity, with EC_{50} values of 0.32±0.05 mg/ml for the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging potential and EC_{50} values of 0.8±0.2 mg/ml for the reducing power. Overall, these results suggest that *Thymus citriodorus* can be a good source of natural antioxidants.

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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).

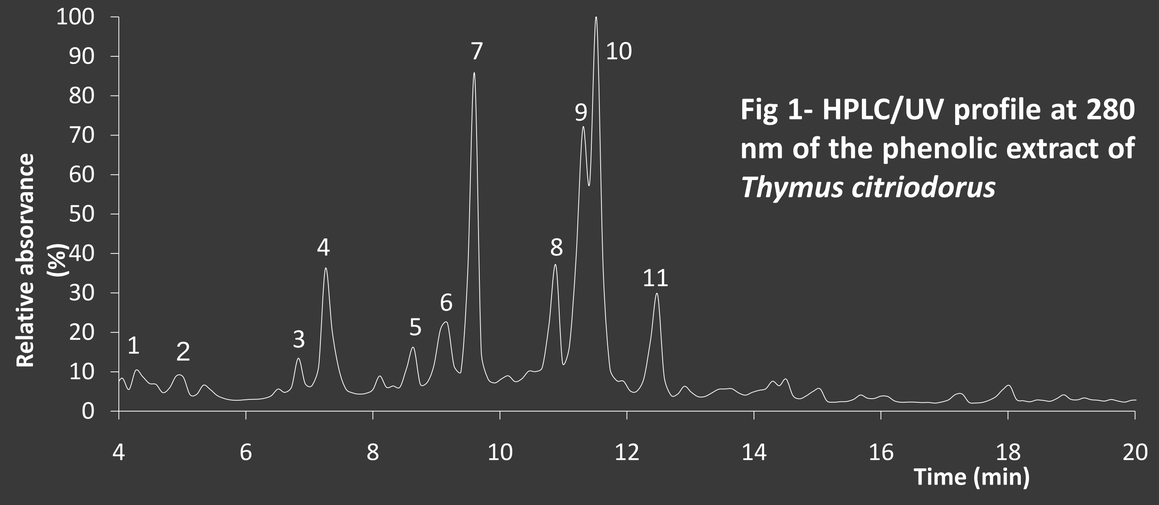

Fig 1- HPLC/UV profile at 280 nm of the phenolic extract of *Thymus citriodorus*

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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