Thymus citriodorus as a source of antioxidants

O Pereira, A M Peres, M R M Domingues, S Cardoso
1DTDT, Escola Superior de Saúde, Instituto Politécnico de Bragança, Portugal,
2Departamento de Fisiologia e Farmacologia, Universidade de Salamanca, Spain,
3CERNAS - Escola Superior Agrária, Instituto Politécnico de Coimbra, Portugal,
4CIMO - Escola Superior Agrária, Instituto Politécnico de Bragança, Portugal,
5LSRE - Escola Superior Agrária, Instituto Politécnico de Bragança, Portugal,
6Centro de Espectrometria de Massa, Departamento de Química, Universidade de Aveiro, Portugal

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 antioxidant capacity, with EC50 values of 0.32±0.05 mg/ml for the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging potential and EC50 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.


Keywords: Thymus citriodorus, phenolic compounds, antioxidant activity, HPLC
THYMUS CITRIODORUS AS A SOURCE OF ANTIOXIDANTS

O. Pereira,1,2,3*, A.M. Peres,5, M.R.M. Domingues,6, S. M. Cardoso,1,4

1QTD, Escola Superior de Saúde, Instituto Politécnico de Bragança, Portugal; 2Departamento de Fisiologia e Farmacologia, Universidade de Salamanca, Espanha; 3CIEMAT - CSIC Contacto: Escola Superior Agrária, Instituto Politécnico de Bragança, Portugal; 4CIMA - Escola Superior Agrária, Instituto Politécnico de Bragança, Portugal; 5Centro de Espectrometria de Massa, Departamento de Química, Universidade de Aveiro, Portugal

*oliviapereira@ipb.pt

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.

RESULTS AND DISCUSSION

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

<table>
<thead>
<tr>
<th>Mass (% of dry weight)</th>
<th>Total Phenolics* (mg/g fraction)</th>
<th>DPPH [EC50] (mg/mL)</th>
<th>Reducing Power [EC50] (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.1</td>
<td>139±14</td>
<td>0.32±0.05</td>
<td>0.8±0.2</td>
</tr>
</tbody>
</table>

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

The phenolic compounds account for 13.9% of the ethanolic extract total and this extract exhibited a high antioxidant capacity, with EC50 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).

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.

REFERENCES


ACKNOWLEDGEMENT

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

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 MS3 [3]. The HPLC analysis was performed on a RP-C18 column 250 mmx 4 mm id, 5μm bead diameter (Temperature of 30°C, 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-picylhydrazyl radical (DPPH) scavenging potential [4] and its reducing power [5].

![Fig 1: HPLC/UV profile at 280 nm of the phenolic extract of Thymus citriodorus](image-url)