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Thymus citriodorus: phenolic characterization and antioxidant activity

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Abstract. The present work aims to determine the phenolic composition of *Thymus citriodorus* and to estimate its antioxidant capacity. An ethanolic extract was prepared from the plant and its total phenolic and flavone contents were determined. The phenolic extract was fractionated by reversed-phase HPLC and the major phenolic compounds of each fraction were identified by ESI-MS and MS^n analysis, in order to establish the specific phenolic profile of the plant.

The total phenolic compounds in the ethanolic extract of *Thymus citriodorus* accounted for 138.75±13.56 mg/g, and its content of flavones was estimated as 27.30±2.15 mg/g. Moreover, the interpretation of the fragmentation pathways under ESI-MS/MS of the collected HPLC fractions allowed concluding that the major phenolic compounds in the plant included some glycoside derivatives of luteonin, naringenin and eriodictyol. Also, the phenolic extract showed a considerable antioxidant activity, as evaluated by the 2,2-diphenyl-1-picrylhydrazyl radical scavenging potential and the reducing potential, suggesting that *Thymus citriodorus* can be an important source of natural antioxidants.

Introduction. *Thymus citriodorus*, also known as Lemon thyme, is a *Lamiacea* subshurb aromatic and medicinal plant cultivated in the Mediterranean region. This plant is frequently used as a deodorant, antiseptic and antifungal element, as well as in the treatment of asthma and other respiratory diseases [1]. It is believed that a part of beneficial activities are due to the volatile constituents 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, such as their phenolic compounds.

Materials and Methods. The phenolic compounds of *Thymus citriodorus* were obtained by extraction with aqueous ethanol (80%). The total phenolic compounds of the ethanolic extract were determined by an adaptation of the Folin-Ciocalteau 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 MS^n. 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].

Results and Discussion. The total amount of phenolic compounds in the ethanolic extract of *Thymus citriodorus* accounted for 138.75±13.56 mg/g and its content of flavones/flavonols was estimated as 27.30±2.15 mg/g. Also, it exhibited a high antioxidative capacity, with EC_{50} values of 0.32±0.05 mg/ml and 0.77±0.15 mg/ml, for scavenging potential and reducing power, repectively.