Antioxidant activities of plants enriched in rosmarinic acid

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ABSTRACT

The present study aims to evaluate the *in vitro* antioxidant potential of *Lavandula dentata* and *Mentha aquatica* plant extracts. For that, ethanolic extracts of the two plants were prepared and their phenolic composition was determined through combined methods of HPLC-DAD and ESI-MS. Moreover, the antioxidant activity of the plant extracts was estimated by: i) evaluation of DPPH scavenging potential and ii) monitoring the protective effects against the generation of reactive oxygen species (ROS) induced by potassium dichromate in human hepatoblastoma HepG2 cells.

M. aquatica ethanolic extract was much enriched in phenolic compounds, in comparison with that of *L. dentata*. Both extracts contained rosmarinic acid in similar concentrations but *M. aquatica* also contained significant amounts of other phenolics, including rutinoside derivatives of eriodictyol and luteolin. The plant extracts showed high radical scavenging activity against DPPH radical and significantly diminished intracellular ROS production under oxidative stress conditions. The latter protection was mostly evidenced in the *L. dentata* extract and was also observed for the rosmarinic acid used as reference.

Attending that rosmarinic acid is a major phenolic component of *L. dentata* and *M. aquatica* ethanolic extracts, the present results suggest that this phenolic compound can be involved in the antioxidant properties of both plants.

1. INTRODUCTION

Lamiaceae plants have been consumed by centuries due to their health benefits, however, the exact composition, as well as the mechanism of action underlying their bioactivities remain, in most cases, unclear [1].

Mentha aquatica L., also known as water mint, is a perennial herb that grows in humid places and it is distributed in temperate regions from Europe and tropical Africa [2]. Previous studies on this plant have attributed it antioxidant activities, both in chemical systems and in cell

models, as well as neuroprotective and anti-inflammatory properties [3, 4, 5]. Some of these effects can be associated to its phenolic constituents, as the plant contains important bioactive polyphenols, such as caffeic and rosmarinic acids, glycosidic derivatives of luteolin and apigenin. In particular, high radical scavenger activity has been demonstrated for some of these compounds [6].

French lavender, or *Lavandula dentata* L., is an aromatic herbaceous small shrub plant native to the Mediterranean region that has been used in folk medicine as an antidiabetic agent and in cold and renal colic treatments [7]. The French lavender has been described to contain luteolin, OH-luteolin 7-*O*-glycoside, scutellarein-7-*O*-glycoside, vitexin, apigenin and genkwanin and glycosidic forms of luteolin, as well as apigenin [8].

2. MATERIALS AND METHODS

2.1 Preparation of plant extracts and phenolic composition assessment

The purified ethanolic extracts of *M. aquatica* and *L. dentata* were obtained by extraction with an 80% ethanolic solution (*v/v*) and further purification on Strata SPE C18-E cartridges, as previously described [9]. The total phenolic amount of the purified extracts was evaluated by the Folin Ciocaulteu assay [10].

The individual phenolic composition of the purified extracts was established by means of HPLC-DAD and ESI-MS combined methods. The HPLC was performed on a Varian 9010 separation module equipped with a PDA Varian Prostar detector. The column used was a 250 mm × 4 mm id, 5 μm bead diameter, end-capped Nucleosil C18 (Macherey-Nagel) (temperature of 30ºC, flow rate of 1 mL/min) and the mobile phase comprised (A) 0.1% formic acid in water and (B) acetonitrile. The phenolic compounds were identified by ESI-MS and MS^n analyses in the negative ion mode, through direct injection of the collected HPLC fractions into the ESI source at a flow rate of 8 μL min^{-1}. Typical ESI conditions were similar to those previously described [9]. The quantification of individual polyphenols was performed at 280 nm by HPLC-DAD using the external standard method.

2.2 Antioxidant activity

The antioxidant activity was determined by in vitro measurement of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging potential [11] and by assessment of the ability to diminish the intracellular ROS incremented production, as induced by potassium dichromate (5 and 25 μM) in human hepatoblastoma HepG2 cells. ROS generation was measured by flow cytometry using dichlorofluorescein diacetate, after 48 h of co-incubation of potassium dichromate and the plant extract or rosmarinic acid [12].

3. RESULTS AND DISCUSSION

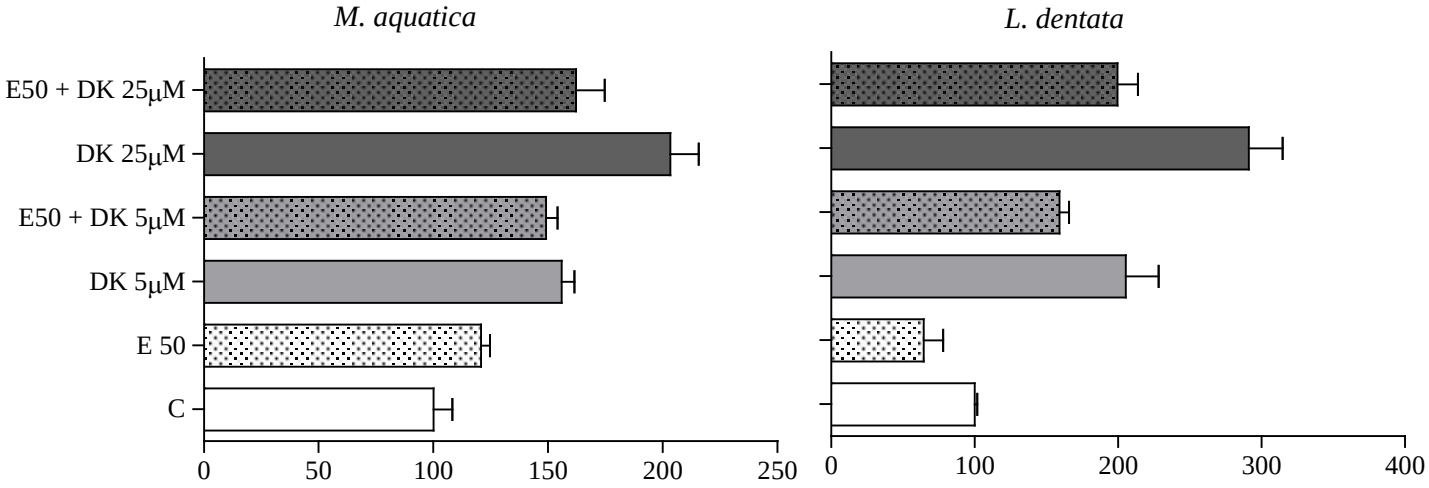
3.1 Polyphenols of *M. aquatica* and *L. dentata* purified extracts

The total amount of polyphenols in the purified extracts of *M. aquatica* and *L. dentata* were 261.8 ± 21.8 GAE mg/g of extract and 174.72 ± 6.89 GAE mg/g of extract, respectively. The extracts contained a similar rosmarinic acid content (67.8±6.7 and 64.2±8.8 mg/g in *L. dentata* and *M. aquatica*, respectively), but contrarily to *L. dentata*, the *M. aquatica* purified extract also contained significant amounts of other phenolics, including eriodictyol-7-*O*-rutinoside and luteolin-7-*O*-rutinoside. Overall, rosmarinic acid accounted for 21% and 72% of the total quantified phenolic compounds, for *M. aquatica* and *L. dentata*, respectively.

3.2 Antioxidant capacity

The concentrations of *L. dentata* and *M. aquatica* extracts able to decrease 50% the DPPH absorbance (EC_{50}) were respectively 11.6±1.1 and 9.5±2.0 µg/mL and hence, the two extracts can be regarded as good radical scavenging agents. In turn, the exposure of HepG2 cells to the non-toxic concentration 50 µg/mL of *M. aquatica* and *L. dentata* extracts resulted in a decreased rate of ROS production under oxidative stress conditions (Fig. 1). This protection was more evident in *L. dentata* extracts, which reduced the ROS production by about 30%. This effect was observed both in basal conditions and in potassium dichromate (5 or 25µM) treated cells. ROS production protection (of about 50%) was also observed in parallel assays performed with rosmarinic acid at 50 µg/mL (data not shown), suggesting that this phenolic acid can have an important role in the antioxidant properties of the *M. aquatica* and *L. dentata*.

Figure 1. Effect of *M. aquatica* and *L. dentata* in ROS incremented production induced by 5 and 25 µM of potassium dichromate in human hepatoblastoma HepG2 cells.



Cells were incubated in the absence (□) or presence of potassium dichromate at 5 (■) or 25 µM (■), co-incubated with 50 µg/ml of the indicated extract (E50), for 48 h.
Values are represented as means ± S.E.M. of percentage of ROS production respect to control, from three different cultures carried out in triplicate per data point. Statistical analysis was performed by one-way ANOVA, followed by Dunnett's post test. DK, potassium dichromate; E, extract; * $P < 0.05$; ** $P < 0.01$

4. CONCLUSIONS

These *in vitro* experiments emphasized the high antioxidant activity of *M. aquatica* and *L. dentata* extracts in chemical and in human hepatoblastoma HepG2 cell models. Overall, the results suggest that rosmarinic acid is associated to the antioxidant properties of these two extracts. Nonetheless, further investigation is required in order to fully understand the mechanisms engaged in the antioxidant properties of these two plants.

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