Anti-inflammatory and antioxidant properties of chemical constituents of *Broussonetia papyrifera*

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Introduction

Broussonetia papyrifera (L.) L'Hér. ex Vent. (Moraceae), commonly known as paper mulberry, is a rich source of bioactive phenolic substances such as coumarins, lignans, 1,3-diphenylpropanes, chalcones, flavans, or flavonols, especially those containing a prenyl group [1,2]. Paper mulberry currently attracts researchers as a source of anti-inflammatory agents. Therefore, as a part of an ongoing investigation of anti-inflammatory agents obtained from plants of the family Moraceae and in connection with a recently published comprehensive review that summarized the anti-inflammatory effects of prenylated phenolic compounds [3], the branches and twigs of *B. papyrifera* have been subjected to extensive chromatographic separation to isolate analogous compounds as potential lead substances to suppress inflammation as well as oxidative stress.

Fig. 1. Branches of *Broussonetia papyrifera* (L.) L'Hér. ex Vent.

Extraction, isolation and elucidation of structures

The branches and twigs of *B. papyrifera* (L.) L'Hér. ex Vent. were collected during May 2017 in the greenhouse of the Faculty of Pharmacy, Masaryk University (MU), Brno, Czech Republic. The air-dried and chopped branches and twigs of *B. papyrifera* (3.2 kg) were extracted with 96% EtOH (3 × 24 h) at room temperature using ultrasonication to support the extraction process. The solvent was removed using a rotavapor to obtain 143 g of crude extract that was partitioned consecutively with *n*-hexane, $CHCl_3$, and EtOAc. The $CHCl_3$-soluble extract (30.9 g) was subjected to silica gel CC (*n*-hexane:$CHCl_3$:MeOH, 10:80:10, v/v/v) to afford twenty-three fractions (BP-1 to BP-23). Fraction BP-7 was subjected to silica gel CC ($CHCl_3$:EtOAc, 85:15, v/v) to yield twelve subfractions (BP-7-A to BP-7-L). Subsequently, selected subfractions were purified by means of preparative HPLC with an Ascentis RP-Amide column (250 mm × 10 mm, 5 µm) using a further defined mixture of MeCN and 0.2% HCOOH (5 mL/min). Extensive separation led to the isolation of thirty compounds, including a novel 5,11-dioxabenzo[b]fluoren-10-one derivative named broussofluorenone C (**12**). The isolated compounds were characterized based on their NMR and HRMS data, and their absolute configurations were established by a combination of NMR, optical rotations, electronic circular dichroism (ECD), and comparison with the data in the literature. In addition, the absolute configurations of furanocoumarins **1** and **2** were unambiguously determined by single-crystal X-ray crystallography.

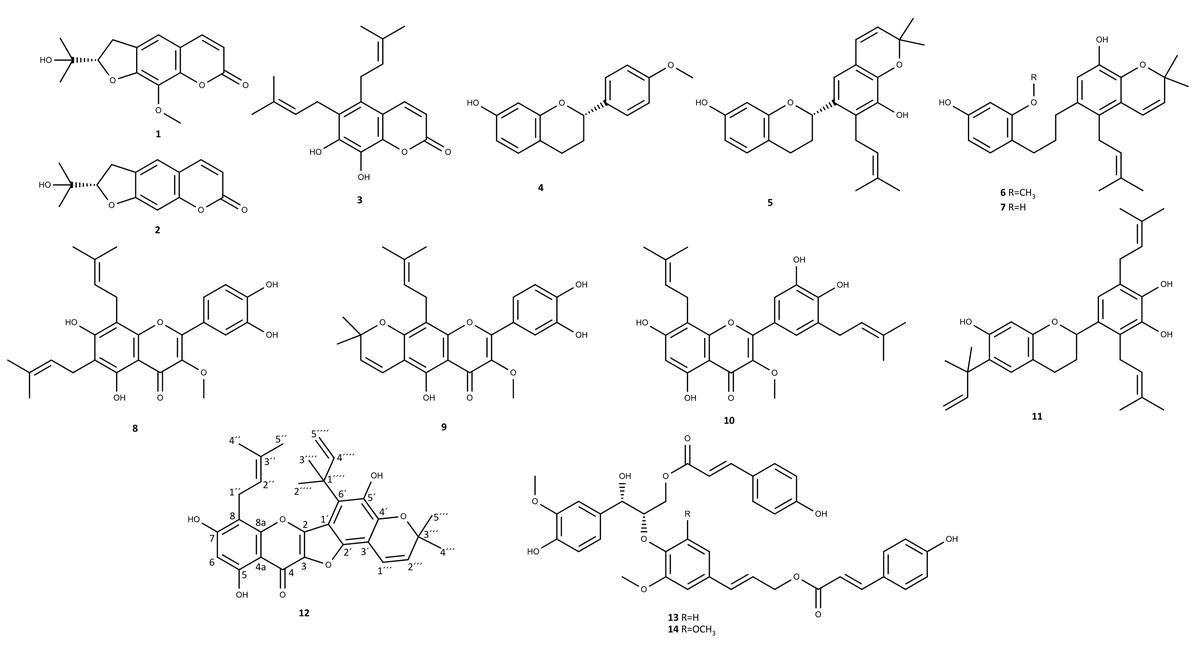

Fig. 2. Structures of the compounds **1**–**14** isolated from branches and twigs of *B. papyrifera*

Anti-inflammatory potential in cell-based models and cellular antioxidant activity (CAA) assay

Selected fourteen structural analogues (**1**–**14**) of previously reported anti-inflammatory active compounds were selected for evaluation of their ability to attenuate the activity of NF-κB/AP-1 in LPS-stimulated THP-1 macrophages and their cellular antioxidant activity.

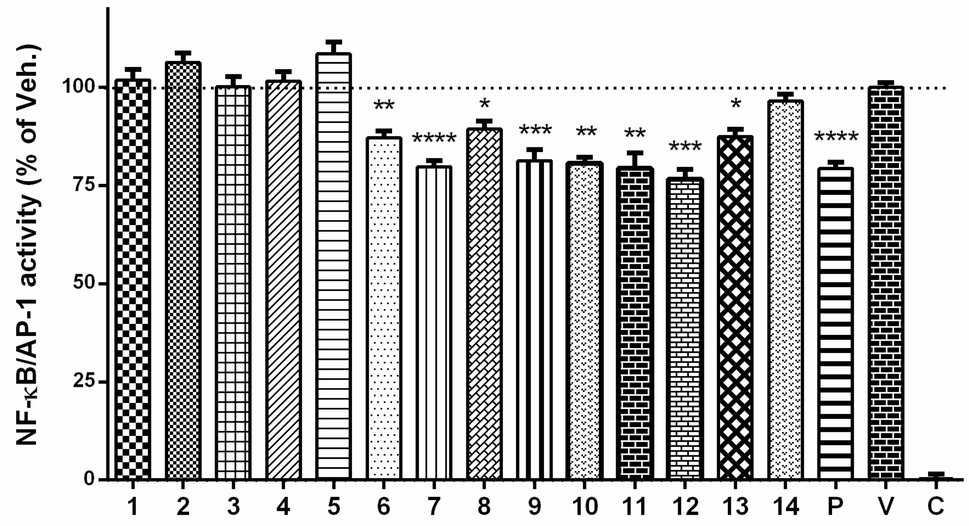

Fig. 3. Inhibitory effects of compounds **1**–**14** at a concentration of 1 µM on the NF-κB/AP-1 activity in THP-1-XBlue™-MD2-CD14 cells stimulated with 1 µg/mL of LPS. Prednisone at a concentration of 1 µM was used as a positive control (P). DMSO, the solvent used for both the test compounds and prednisone, was added to the vehicle control (V) and to the non-stimulated cells (C).

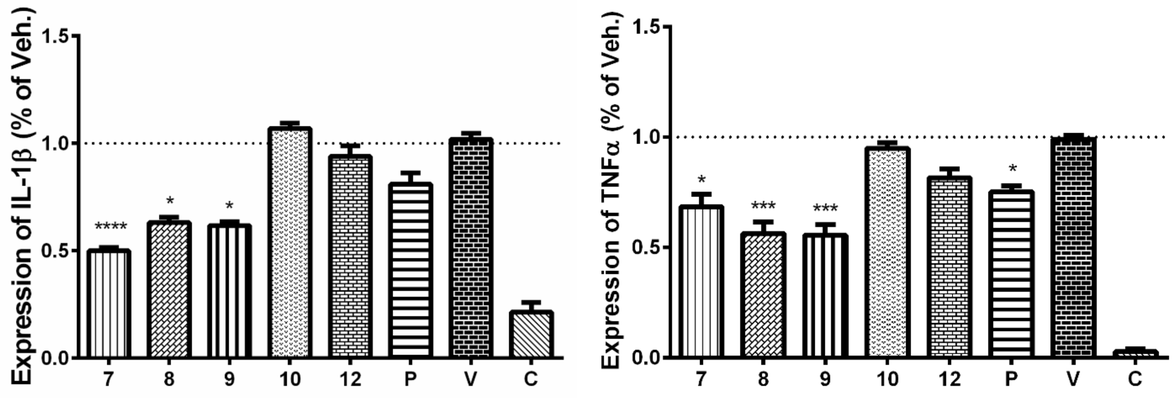

Fig. 4. Inhibitory effects of compounds **7**–**10** and **12** at a concentration of 1 µM on the secretion of IL-1β and TNF-α in THP-1-XBlue™-MD2-CD14 cells stimulated with 1 µg/mL of LPS. Prednisone at a concentration of 1 µM was used as a positive control (P). DMSO, the solvent used for both the test compounds and prednisone, was added to the vehicle control (V) and to the non-stimulated cells (C).

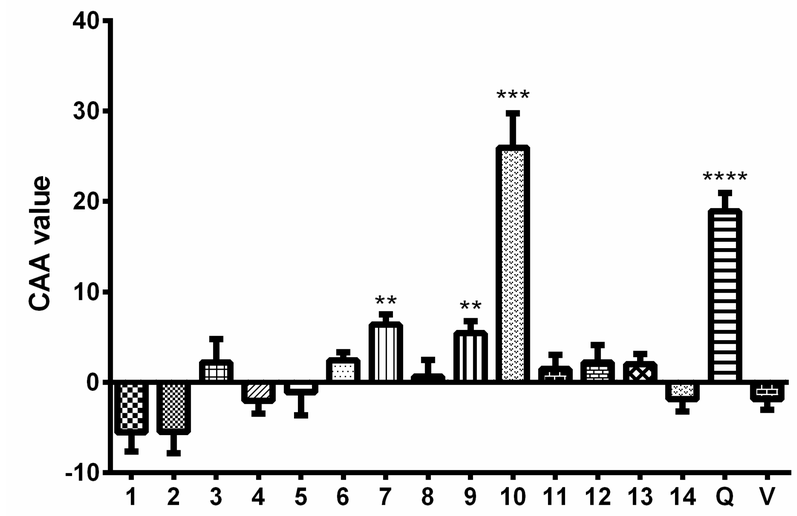

Fig. 5. Antioxidant activity of compounds **1**–**14** at a concentration of 5 µM in a CAA assay in THP-1 cells. Quercetin at a concentration of 5 µM was used as a positive control (Q). DMSO, the solvent used for both the tested compounds and quercetin, was added to the vehicle control (V).

Discussion and conclusion

In summary, thirty compounds have been identified, including a novel 5,11-dioxabenzo[b]fluoren-10-one derivative (**12**). Subsequently, the anti-inflammatory and antioxidant activities of selected isolated compounds **1**–**14** have been investigated. Chemical constituents **7**–**9** and **12** were demonstrated to be potent anti-inflammatory agents with moderate antioxidant activity, while compound **10** exhibited significant antioxidant effect but did not affect the secretion of pro-inflammatory cytokines. Compounds **7**–**9** showed the ability to inhibit NF-κB signaling in the THP-1 cell line as well as to inhibit the production of the pro-inflammatory cytokines TNF-α and IL-1β. Among these, compounds **7** and **9** were able to reduce the production of ROS in THP-1 cells. Considering the results, *B. papyrifera* deserves more attention in connection with its bioactive constituents and could be cultivated more extensively for both industrial and medicinal purposes.

References:

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