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Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of small yellow onion (*Allium flavum* L. subsp. *flavum*, Alliaceae)

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ABSTRACT

The objectives of this study were to define the phenolic profile, antioxidant, anti-inflammatory and cytotoxic properties of edible *Allium flavum* subsp. *flavum* (small yellow onion), which has never been comprehensively examined before. The presence and content of 44 phenolic compounds in methanol extracts of *A. flavum* were investigated by LC-MS/MS, where 25 compounds were found, the most dominant being: ferulic, *p*-coumaric, caffeic, *p*-hydroxybenzoic, vanillic, protocatechuic and syringic acid, rutin, quercetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside. Antioxidant activity, determined through several assays, was low in comparison to the synthetic antioxidant butylated hydroxytoluene, but comparable to onion (*Allium cepa* L.) extract. Anti-inflammatory potential was studied by measuring the inhibitory effect on cyclooxygenase-1 (COX-1) and 12-lipoxygenase (12-LOX) activity, where *A. flavum* expressed high inhibitory potential, especially on 12-LOX activity (IC_{50} = 0.078 mg mL^{-1}). Treatment of four human cell lines resulted in a considerable inhibition of cell growth, where the extract of *A. flavum* expressed selective inhibitory action towards cervix epithelioid carcinoma and colon adenocarcinoma cells (IC_{50} = 71 μg mL^{-1} and IC_{50} = 81 μg mL^{-1}, respectively). To conclude, these results support use of *A. flavum* as a functional food and indicate that it could be a potent source of health-beneficial phytochemicals.

1. Introduction

Allium flavum subsp. *flavum* is a member of the genus *Allium*, which is by far the largest genus of the Alliaceae family, comprising about 750 species. Some of the species represent the underlying taxa for cultivated forms, which are widely used in human diet as spices and vegetables (onion – *Allium cepa* L., garlic – *Allium sativum* L., leek – *Allium porrum* L.). Considering also the high biological activity of these well-researched cultivated species, we found it worthwhile to investigate the chemical composition and biological activities of an unexplored member of the genus *Allium* – a wild-growing *A. flavum* subsp. *flavum*. This is a deciduous plant with simple leaves and yellow flowers, known by the common name "small yellow onion". Leaves and bulbs are edible, are of a milder taste and smell than onion and are used traditionally in Balkan region as a spice for soups, stews and salads (Grlic, 1986). *A. flavum* is native to South, Central and Eastern Europe and Central Asia (Anackov, 2009).

There have been very few published data on either its chemical constituents or biological potential. Besides a taxonomic study of sulfoxide compounds in this species (Kusterer, 2010), there are no other data on the chemical profile of this species. Furthermore, there are only a few biochemical reports confirming that *A. flavum* extracts possess a high antioxidant (Curcic et al., 2012; Stajner, Igic, Popovic, & Malencic, 2008), antibacterial and antiproliferative activity (Curcic et al., 2012), as well as anti-*Aspergillus* properties

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(Yin & Tsao, 1999). However, there have been no available reports on anti-inflammatory activity of this species. Considering that *A. flavum* is traditionally used as a food, we considered it important to examine whether this species could be regarded as functional food possessing both nutritional and healthy properties.

Therefore, the aim of the present study was to explore the phenolic profile and anti-inflammatory potential of *A. flavum* subsp. *flavum*, as well as to expand knowledge of their antioxidant and antiproliferative properties and to compare these biological activities with the activities of *A. cepa*, the official drug (WHO, 1999).

2. Materials and methods

2.1. Plant material and extract preparation

The whole plants of wild-growing *A. flavum* L. 1753 subsp. *flavum* var. *flavum* f. *flavum* were collected in July 2009 from three different locations in Serbia (Vrsacki breg, Dimitrovgrad, Babusnica). Samples of the grown onion (*A. cepa* L.) were collected also in July 2009 in the village of Neradin, the Fruška Gora Mountain, Serbia. The voucher specimens (*A. flavum* subsp. *flavum*, Vrsacki breg – no. 2-1769, Dimitrovgrad – no. 2-1765, Babusnica – no. 2-1767; *A. cepa*, no. 2-1762) were prepared, identified and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), University of Novi Sad, Faculty of Sciences.

30 g of air-dried and ground plant material (whole plants, aerial parts, bulbs) were macerated with 70% aqueous methanol (8 mL per 1 g of dw) during 72 h at 30 °C. After filtration, the solvent was evaporated to dryness under vacuum at 45 °C and dry residues were re-dissolved in 70% aqueous methanol to the final concentration of 300 mg mL^{-1} (for antioxidant assays) or in DMSO to obtain 300 mg mL^{-1} stock solutions (for evaluation of anti-inflammatory and cytotoxic activity). Prepared extracts (300 mg mL^{-1} in 70% aqueous methanol) were diluted with a mixture of 0.5% aqueous formic acid and methanol (in ratio of 7:3) to obtain 2 mg mL^{-1} stock solutions for LC-MS–MS analysis of the phenolic profile.

2.2. Quantitative LC-MS/MS analysis of the selected phenolics

The content of quinic acid and 44 selected phenolic compounds (14 phenolic acids, 25 flavonoids, 3 coumarins and 2 lignans) was investigated by LC-MS/MS according to the previously reported method (Beara et al., 2012). Standards of the compounds were purchased from Sigma–Aldrich Chem (Steinheim, Germany), Fluka Chemie GmbH (Buchs, Switzerland) or from ChromaDex (Santa Ana, USA).

The Agilent 1200 series liquid chromatograph, coupled with Agilent series 6410B electrospray ionization triple-quadrupole mass spectrometer and controlled by MassHunter ver. B.03.01. software, was used for analysis. Analytes were separated using a Zorbax Eclipse XDB-C18 4.6 mm × 50 mm × 1.8 μm (Agilent Technologies) reversed-phase column. Compound-specific, optimized MS/MS parameters are given in Table 1.

2.3. Antioxidant activity

Antioxidant potential was determined using several assays: assays related to free radical ($DPPH^{\bullet}$, $ABTS^{\bullet+}$), reactive oxygen ($HO^{\bullet}$) and reactive nitrogen species ($NO^{\bullet}$) scavenging activity, and ability to inhibit lipid peroxidation (LP). The synthetic antioxidant butylated hydroxyanisole (BHA) was used as a positive control. $ABTS^{\bullet+}$ scavenging activity was examined by using the Total Antioxidant Status kit (Biorex Diagnostics Limited, Antrim, UK), inline with the specified recommendations. The total antioxidant status (TAS) of the extract is expressed in mmol of Trolox equivalents per gram of dry weight. DPPH radical scavenging activity was measured according to the method described in Beara et al. (2012). NO scavenging capacity and LP inhibition ability were determined by methods published by Orcic, Mimica-Dukic, Franciskovic, Petrovic, and Jovin (2011). $HO^{\bullet}$ scavenging activity was measured using the ESR DMPO spin trap method (Babovic et al., 2010). ESR spectra were recorded after 2.5 min, with the following spectrometer settings: field modulation 100 kHz, modulation amplitude 0.226 G, receiver gain 5 × 105, time constant 80.72 ms, conversion time 327.68 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, and temperature 23 °C.

2.4. COX-1 and 12-LOX inhibition assays

Anti-inflammatory potential was studied by e*x vivo* COX-1 and 12-LOX assay previously described by Beara et al. (2010) and modified by Lesjak et al. (2013). Estimated IC_{50} values were compared to IC_{50} values of standards – aspirin (acetylsalicylic acid) (Sigma Aldrich, Germany), a well-known COX-1 inhibitor, and quercetin (Sigma Aldrich, Germany), a 12-LOX inhibitor.

2.5. Effect on cell growth

Antiproliferative activity was evaluated *in vitro* by the estimation of cell growth effects in four human cell lines: HeLa (cervix epithelioid carcinoma; ECACC No. 93021013), MCF7 (breast adenocarcinoma; ECACC No. 86012803), HT-29 (colon adenocarcinoma; ECACC No. 91072201) and MRC-5 (human fetal lung; ECACC No. 84101801). Cell lines were grown in DMEM (PAA Laboratories GmbH, Pashing, Austria) with 45 mg mL^{-1} glucose, supplemented with 100 μL mL^{-1} heat inactivated FCS (PAA Laboratories GmbH, Pashing, Austria), 100 IU mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin. They were cultured in 25 cm^2 flasks at 37 °C in atmosphere of 5% CO_2 and high humidity, sub-cultured twice a week and a single cell suspension was obtained using 1 mg mL^{-1} trypsin with 0.4 mg mL^{-1} EDTA.

Extracts were diluted in 9 mg mL^{-1} NaCl and sterilized by filtration through 0.22 μm micro filters (Sartorius, Germany). Serial dilutions of extracts (20 μL per well) were added in 180 μL of medium to achieve the required final concentrations. Serial dilutions of standards in DMSO (1 μL) were added in 199 μL of medium. Equal volumes of solvents were added in control wells. Concentration of DMSO in cell culture was $\leq$5 μL mL^{-1}.

Cell growth was evaluated by Sulforhodamine B (SRB) assay previously published by Cetojevic-Simin et al. (2012).

3. Results and discussion

3.1. LC-MS/MS analysis of the selected flavonoids

The phenolic profile of *A. flavum* extracts has not been investigated so far. In this study, 44 plant phenolics and quinic acid (an intermediate in plant phenolics biosynthesis) were quantified in aerial parts and bulb extracts of *A. flavum* from the three locations in Serbia, by using the LC-MS/MS technique. The MRM mode was applied as the preferred acquisition method for the accurate quantification. This type of analysis provides the high sensitivity and specificity, due to the fact that only ions specific to targeted analytes are monitored. Representative chromatograms are shown in Fig. 1, while the overall data concerning the content of the phenolic compounds are presented in Table 2. The results of the analysis showed that the aerial parts extracts of *A. flavum* are rich in phenolic acids and flavonoids (25 compounds were detected), while the bulb