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Mitodepressive, antioxidant, antifungal and anti-inflammatory effects of wild-growing Romanian native *Arctium lappa* L. (*Asteraceae*) and *Veronica persica* Poiret (*Plantaginaceae*)

Radu Claudiu Fierascu[a,b], Milen I. Georgiev[a,c], Irina Fierascu[a,b,*], Camelia Ungureanu[d], Sorin Marius Avramescu[a,e], Alina Ortan[a], Mihaela Ioana Georgescu[a], Anca Nicoleta Sutan[f], Anca Zanfirescu[g], Cristina Elena Dinu-Pirvu[a,g], Bruno Stefan Velescu[g], Valentina Anuta[g]

[a] University of Agronomic Science and Veterinary Medicine, Bucharest, Romania
[b] The National Institute for Research & Development in Chemistry and Petrochemistry, ICECHIM, Bucharest, Romania
[c] Laboratory of Applied Biotechnologies, Institute of Microbiology, Bulgarian Academy of Sciences, Plovdiv, Bulgaria University, Romania
[d] Politehnica of Bucharest, Faculty of Applied Chemistry and Material Science, Bucharest, Romania
[e] Research Center for Environmental Protection and Waste Management, University of Bucharest, Bucharest, Romania
[f] University of Pitesti, Department of Natural Sciences, Pitesti, Arges, Romania
[g] Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

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ABSTRACT

The present study aims to evaluate the potential uses of hydroalcoholic extracts obtained from Romanian native wild-growing plants. The hydroalcoholic extracts were obtained from the burdock roots and respectively the aerial parts of birdeye speedwell. The extracts were characterised by HPLC (quantifying 13 compounds in the *V. persica* extract, 6 compounds in the *A. lappa* extract and confirming the presence of arctiin and arctigenin in the burdock extract). The antioxidant potential of the crude extracts was evaluated using two methods: the DPPH assay (79.91% for speedwell extract, 76.23% for burdock extract) and the phosphomolybdate method (296.5 mg/g ascorbic acid equivalents for burdock, 324.4 mg/g for speedwell). The crude extracts were found to be active against both fungal lines used (*Aspergillus niger* and *Penicillium hirsutum*), inhibition zones - 17.1 mm and 13.1 mm against *P. hirsutum*, respectively ca. 22 mm for both extracts against *A. niger*. The cytogenetic effects (assessed using the *Allium cepa* assay) revealed a series of chromosomal aberrations and nuclear aberrations induced in the meristematic root cells. The anti-inflammatory effect, estimated in two inflammation experimental models, showed a significant effect, especially for the speedwell extract. The results recommend the evaluated extracts as promising sources of biologically-active compounds.

1. Introduction

Since ancient times, medicinal plants have been used for the treatment of a tremendous array of diseases and disorders, ranging from common colds to more serious conditions, or as antifungal or insect repellent tools (Kristanc and Kreft, 2016). However, despite the progress made in pharmaceutical and especially drug-discovery science, new uses of medicinal plants and new natural occurring compounds are proposed for medical applications (Yu et al., 2017).

The two medicinal plants considered within the present study, *Arctium lappa* L. (common name burdock) and *Veronica persica* Poiret (commonly referred as birdeye speedwell) have a very long track record of use in Romanian folk medicine, most often belonging to the wild-flora, as weeds. They were selected for the present study considering two main aspects: on one hand their common anti-inflammatory potential and, on the other hand, their underuse and lack of scientific studies on their applications. Burdock is traditionally applied for both internal (in liver diseases, as diuretic or hypoglycaemic) or external (for the treatment of eczema and skin infections, just to name a few) treatment (Bojor, 2003). Therapeutic activity has been attributed to flavonoids and lignans content of burdock roots. It has been reported that antioxidative, anti-inflammatory and hepatoprotective effects are due to the caffeoylquinic acid derivatives (Maruta et al., 1995; Lin et al., 1996) and that antiproliferative and apoptotic effects are

* Corresponding author. University of Agronomic Science and Veterinary Medicine, Bucharest. Romania; The National Institute for Research & Development in Chemistry and Petrochemistry, ICECHIM, Bucharest, Romania.
E-mail address: dumitriu.irina@yahoo.com (I. Fierascu).

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attributable to the presence of arctigenin (Matsumoto et al., 2006) and lappaol A, C and F (Ming et al., 2004).

In contrast to burdock, birdeye speedwell is a very common, short, sprawling, annual herb. Recent studies have evaluated applications of extracts or purified compounds from burdock roots as adjuvant anticancer agents (Su et al., 2015), antitubercular (Zhao et al., 2014), anti-inflammatory intestinal agents (de Almeida et al., 2013), neuroprotective (Tian et al., 2014), acne treatment (Miglani and Manchanda, 2014), as well as the anti-inflammatory potential (Vogl et al., 2013), antibacterial, antioxidant (Stojković et al., 2013; Mocan et al., 2015), antimutagenic and antitumor potential (Zivkovic et al., 2014) of different types of *Veronica* species. Pharmacological and biological activities of *Veronica* species could be attributed to their content in secondary metabolites such as iridoids, phenolic acids and flavones. Previous studies showed that *V. persica* is rich in iridoids, especially aucubin and catalpol type (Crişan et al., 2010).

The present work describes the prospective applications of burdock roots and birdeye speedwell aerial parts hydroalcoholic extracts for anticancer applications, treatment of different inflammatory and fungal diseases, as well as adjuvant antioxidant agent, by studying their mitodepressive, anti-inflammatory, antifungal, and antioxidant activity.

Establishing the biological activities of natural products extracts is dependent on the understanding of their *in vivo* intake, absorption, metabolism and excretion. Although polyphenols have shown countless health benefits, they have limited application as pharmaceutical products; even if they are in the form of glycosides and have relatively high polarity, their limited water solubility prevents the passive *in vivo* absorption (Kaur and Kaur, 2014). As a result, their bioavailability is generally low and can vary drastically among different polyphenol classes as well as individual compounds in a particular class (Manach et al., 2004, 2005). Besides poor permeability, many of the polyphenols are subject to extensive gut and liver metabolism (Lewandowska et al., 2013), limiting furthermore the therapeutic outcome of their administration. Therefore, formulation of the resulted plant extracts in drug delivery systems capable to ensure an adequate absorption of the active compounds from the gastrointestinal tract is a necessary step for the *in vivo* evaluation of their efficacy.

2. Materials and methods

2.1. Plant material and extraction conditions

Wild-growing *Veronica persica* L. and *Arctium lappa* L. were identified and harvested from fields in the Dobresti area, Pitesti hills (44°57′48″N, 25°6′58″E). Multiple specimens were collected during summer (July), paying special attention to the harvest of burdock roots not longer than 60 cm; representative voucher specimens were deposited in BUAG Herbarium, Bucharest for future reference (voucher nos. 40006 for birdeye speedwell and 40007 for burdock, respectively).

The crude extracts used for the study were obtained from ground shade-dried plant material (20 g) in 1:1 mixture water-ethanol (100:100 mL), as previously described (Fierascu et al., 2015, 2016), at 80 °C, for 2 h. The experiments were carried out using analytic grade ethanol (Merck KGaA, Germany) and bidistilled water (obtained by a GFL 2102 water still). The plant material represents the aerial part of *V. persica* (flowers, leaves and stem) and roots of *A. lappa*. For the HPLC analyses and for the formulation of microemulsions, the extracts were concentrated on a Heidolph rotary evaporator system.

2.2. Chromatographic evaluation of tested extracts

The chromatographic analyses were carried out using a 1200 series Agilent (Agilent Technologies, Darmstadt, Germany), consisting in a binary pump (G1312B), degasser (G1379B), thermostated automated sample processor (G1329A), column thermostat and DAD detector (G1315B). Data acquisition and processing were performed using Chemstation software (Agilent Technologies). The chromatographic separation was performed using a Phenomenex Kinetex C18 core shell column (150 × 4.6 mm i.d., 5 μm particle size) maintained under constant temperature (30 °C). The mobile phase consisted in a mixture of 0.1% trifluoracetic acid (solvent A) and acetonitrile (solvent B), at 1.0 mL/min flow rate, using the following elution program: 0 min, 3% B; 2 min, 3% B; 15 min, 20% B; 20 min, 20% B; 35 min, 45% B; 40 min, 50% B; 40.1 min, 3% B. All solutions were filtered through a 0.45 μm pore size filter (LABTECH VP30) and degassed by sonication. The injection volume was 5 μL. The detection was set at 210 nm for the iridoid derivatives, 270 nm for hydroxybenzoic acid derivatives, 280 nm for flavanols, flavanones and lignans, 320 nm for the hydroxycinnamic acids and flavones, and 370 nm for flavonols. Spectra were recorded for each compound in the range 190–400 nm. Peak identification was performed based on the retention times, as well as comparison with the UV spectra of the reference compounds. Five-points calibration curves were constructed for each of the analysed compounds ($R^2 > 0.999$) using commercially available reference substances. The catechins [(+)-catechin, (+)-gallocatechin, (−)-epigallocatechin], as well as hyperoside, quercitrin, kaempherol-3-*O*-glucoside and kaempherol-3-*O*-rutinoside were purchased from Extrasynthese (Genay, France), while the other reference standards (gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, quercetin, rutin, myricetin, luteolin, luteolin-7-*O*-glucoside, apigenin, hesperetin, aucubin, catalpol) were acquired from Sigma-Aldrich (St Louis, MO, USA). Since many phenolic compounds are mostly present as glycosides in plants and a limited number of glycoside standards were available, acidic hydrolysis of the extracts (HCl 2N, 2 h at 85 °C, followed by ethyl acetate extraction) was also performed for a better understanding of the relevant aglycones.

2.3. Antioxidant assays

The antioxidant activity of the crude extracts was evaluated using two methods: the DPPH free radical scavenging method and the phosphomolybdate method (evaluation of total antioxidant activity). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed as previously described (Fierascu et al., 2015). The phosphomolybdate method implies the following protocol: to a 0.3 mL sample solution were added 2.7 mL of phosphomolybdate reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixtures were incubated at 95 °C for 90 min. After cooling the samples to room temperature, their extinction was measured at 695 nm on a Unicam Helios α Thermo Orion UV-VIS spectrophotometer. Antioxidant capacity is expressed in ascorbic acid equivalent to 1 mg of active substance. The calibration curve for ascorbic acid is linear in the range of 0.001–1 mg/mL, $n = 5$, $R^2 > 0.994$ (Pandey et al., 2016). All experiments were carried out in triplicate.

2.4. Determination of antifungal effect

The antifungal activity of the crude extracts was tested against two relevant fungal strains *Aspergillus niger* ATCC 15475 and *Penicillium hirsutum* ATCC 52323 by Kirby-Bauer antibiotic testing (KB testing or disk diffusion antibiotic sensitivity testing) (Bauer et al., 1966; Ungureanu et al., 2016).

The moulds were grown on potato-dextrose agar (abbreviated "PDA") from Sigma-Aldrich with the following composition: potato extract, 4 g/L., Dextrose, 20 g/L, agar, 15 g/L. The inhibition zone evaluation was performed according to National Committee for Clinical Laboratory Standards (NCCLS) standards (NCCLS, 2006; Barbinta-Patrascu et al., 2014). The stock culture was kept at 4 °C. Briefly, 20 mL of sterile medium were poured into the Petri dish and the microorganism strain (1 mL) was spread on agar plates; using a sterile Durham tube (6 mm diameter), the wells were prepared according to the number of samples. The wells were inoculated with 50 μL sample. All the test plates were incubated at 37 °C for 144 h, in order to allow