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Original Article

Anti-inflammatory and antioxidant activities of the *Impatiens noli-tangere* and *Stachys officinalis* polyphenolic-rich extracts

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ABSTRACT

This study evaluated the anti-inflammatory and antioxidant activities of *Impatiens noli-tangere* L., Balsaminaceae, and of *Stachys officinalis* L., Lamiaceae, polyphenolic-rich extracts obtained by nanofiltration process. Results showed the great potential and efficiency of the nanofiltration process to concentrate the herbal extract's main polyphenolic compounds (over 91% phenolic acids and flavonoids retention). *S. officinalis* polyphenolic-rich extracts had high antioxidant activities (IC_{50} 2.5 μg/ml) compared to *I. noli-tangere* polyphenolic-rich extracts (IC_{50} 19.3 μg/ml) and similar with that of ascorbic acid. Polyphenolic-rich extracts were investigated to determine the pro-inflammatory enzymes lipoxygenase Q2 (LOX), cyclooxygenase (COX-1 and COX-2) and their inhibitory activity. Furthermore, high inhibitory activity of the examined extracts was reported for the first time, for both LOX (IC_{50} 2.46 and 1.22 μg/ml for *I. noli-tangere* and *S. officinalis* polyphenolic-rich extracts, respectively), COX-1 (IC_{50} 18.4 and 10.1 μg/ml for *I. noli-tangere* and *S. officinalis* polyphenolic-rich extracts, respectively) and COX-2 (IC_{50} = 1.9 and 1.2 mg/ml for *I. noli-tangere* and *S. officinalis* polyphenolic-rich extracts, respectively). Additionally, the *in vivo* studies showed that *S. officinalis* polyphenolic-rich extract has a higher anti-inflammatory effect, the hind-paw volume employed for both models determined that *I. noli-tangere* polyphenolic-rich extract and is also higher than that of diclofenac. It was noticed that their anti-inflammatory effect persists for more than 24 h. The *I. noli-tangere* and *S. officinalis* polyphenolic-rich extracts exert anti-inflammatory and antioxidant activities and these properties can be at least partly assigned to the presence of ursolic acid, caffeic acid, rosmarinic acid, quercetin and also anthocyanidins (genistin). The obtained results indicate the anti-inflammatory potential of the studied herbal extracts.

Introduction

Nowadays, the increasing rate of the inflammatory diseases requires new alternative solutions to the nonsteroidal anti-inflammatory drugs and a wider range of inflammatory products, as well. Inflammation is a normal biological process in response to tissue injury, microbial pathogen infection and chemical irritation. Wound healing is a natural and fundamental histopathological process that restores the function and the integrity of the damaged tissues. This process includes three overlapping phases: hemostasis and inflammation, tissue formation and remodeling (Clark, 1995). Therefore, a healing extract should exhibit anti-inflammatory and antioxidant activities in order to have high efficiency healing. Investigations performed over the last years proved that the Lipoxygenase (LOX) and Cyclooxygenase (COX-1 and COX-2) pathways play an important role in the inflammatory disorder's development. In the same way, the drugs that inhibit LOX concurrently with COX-1 and COX-2 may enhance tissue regeneration (Martel-Pelletier et al., 2003; Hayashi et al., 2011; Steinhilber and Hofmann, 2014). Leukotriene (LTB_4), produced by the oxidation of arachidonic acid by 5-LOX, is known to be an active chemo-attractant for fibroblasts and monocytes and may regulate fibroblast activities in the skin's wound healing (Jovanovic et al., 2001). COX, may also be involved in wound healing because it appears to be associated with directed cell motility and new tissue's growth in airway epithelium, and skin (Savla et al., 2001).

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Antioxidants exert defensive effects against oxidative stress, by scavenging free radicals (Valko et al., 2007). Recent researches findings indicated that plant based antioxidants represent great therapeutic agents to combat oxidative stress closely associated with chronic inflammation (Njenga and Viljoen, 2006). Hence, antioxidants are believed to accelerate wound healing. (Fitzmaurice et al., 2011).

Impatiens noli-tangere L., Balsaminaceae, commonly known as touch-me-not balsam is an annual herbaceous plant found in damp places and forests in Europe, Asia and North America. Plants belonging to the genus *Impatiens* are rich in organic acids, anthraquinones, flavonoids and phenolic acids (Choi and Kim, 2002; Paun et al., 2016).

Stachys officinalis L. (syn. *Betonica officinalis* L.), commonly known as wood betony or Bishop's wort is a perennial herb and is one of the largest genus of the Lamiaceae family (Mabberly, 1997). *S. officinalis* contains various alkaloids, saponins, flavonoids and phenolic acids and has been used in traditional medicine to treat various disorders such as: respiratory tract, gastrointestinal tract, nervous system, inflammatory disease and liver disorders (Háznagy-Radnai et al., 2012; Vogl et al., 2013; Šliumpaite et al., 2013; Rigat et al., 2015).

In Romanian traditional medicine *I. noli-tangere* and *S. officinalis* are known for their antioxidative, anti-inflammatory, astringent, hemostatic and wound healing properties (Ardelean and Mohan, 2008; Vogl et al., 2013; Jarić et al., 2017). The literature provides limited information about the chemical composition and biological activity of *I. noli-tangere*. However, the anti-inflammatory effects of *I. noli-tangere* and *S. officinalis* polyphenolic-rich extracts have not been studied.

Material and methods

Samples and reagents

Phenolic compound standards were purchased from Sigma Chemical Company (Sigma Aldrich, Germany) and Roth (Carl Roth GmbH, Germany), solvents were purchased from Sigma Chemical Company (Sigma Aldrich, Germany) and kaolin was obtained from Health Chemicals Co. Ltd. (China). Polymeric membranes were purchased from Millipore and Sterlitech. *I. noli-tangere* L., Balsaminaceae, and *S. officinalis* L., Lamiaceae, herbs were collected from Cluj region, Romania. The voucher specimen no. 640632/1985 (det. Gh. Groza) for *I. noli-tangere* and voucher specimen no. 658238/2005 (det. L. Filipas) for *S. officinalis* were deposited in the Botanic Garden of Cluj-Napoca.

Animals

All experiments were performed on adult male rats ($n = 32$; 197 ± 48 g), purchased from UMF Biobase, Bucharest. They were housed eight per cage in a ventilated cage fitted with wood sawdust bedding, with free access to water and food pellets. The experiments were performed under controlled light/dark cycle conditions (12 h light/12 h dark; lights on at 6 a.m.) in the temperature ranged between 20 and 22 °C and the RH was maintained at 35–45%. All procedures were carried out according to EU Directive 2010/63/UE and with the approval of the Institutional Animal Care and Use Committee (Approval for pre-clinical experimental research based on the protocol no. HERB07/21.01.2016).

Preparation of polyphenolic-rich fractions from plant sources by nanofiltration

Air-dried leaves and stems of herbs were grounded, extracted with 50% EtOH then introduced in a sonication bath (Elma Transsonic T 460, Germany), at a frequency of 35 kHz for 90 min. The herbal's mass concentration in the solvent was 100 g/l. After filtration through Whatman filter paper, the extracts were microfiltred through 0.45 μm pore size microfiltration membrane (MF) (Millipore) as to remove any fine solid particles. The concentration experiments were further carried out by nanofiltration (NF) membrane (Sterlitech membrane, NF90 with cut-off 200–400 Da).

Membrane filtration experiments were performed using a lab cross-flow membrane filtration unit (KMS Laboratory Cell CF-1, Koch Membrane, Germany). NF experiments were performed according to the batch concentration mode, at a 9 bar transmembrane pressure and at 23 ± 2 °C temperature. The performance of the nanofiltration process was evaluated through the rejection (R, %) of NF membrane toward specific compounds (total phenolic content), calculated as follows:

$$R = \left(1 - \frac{c_p}{c_f}\right) \times 100 \quad (1)$$

where c_p and c_f are the permeate and the feed concentrations of the total phenolics.

Extracts analysis

Determination of the total phenolic content and of the flavonoids

The phenolic total content was determined by the Folin-Ciocalteu method (Singleton et al., 1999), measuring the absorbance at 760 nm, using gallic acid (GAE) as a standard. Total polyphenols contents were obtained from the regression equation of the gallic acid's calibration curve ($y = 0.0032x + 0.0073$, $R^2 = 0.9963$) and expressed as GAE equivalents.

The total flavonoids content was assessed by the aluminum chloride colorimetric assay with the absorbance measured at 430 nm and quercetin (QE) used as standard (Lin and Tang, 2007). Results were expressed as mg QE equivalents/l of extract. Total flavonoid contents were obtained from the regression equation of the quercetin's calibration curve ($y = 0.0051x + 0.0286$, $R^2 = 0.9966$).

HPLC–MS analyses of phenolic, anthocyanidins and anthocyanins compounds

The polyphenol's measurement method was based on the previous HPLC analysis to evaluate the compounds in the extracts (Cristea et al., 2009). The chromatographic measurements were performed using a complete HPLC Shimadzu system, through a C18 Nucleosil 3.5, 4.6 × 50 mm, Zorbax column. The system was coupled to a MS detector, LCMS-2010 detector (liquid chromatography mass spectrometer), equipped with an ESI interface. The mobile phase consists of formic acid in water (pH 3.0) as solvent A and formic acid in acetonitrile (pH 3.0) as solvent B. The polyphenolic compound's separation was performed using binary gradient elution: 0 min 5% solvent B; 0.01–20 min 5–30% solvent B; 20–40 min 30% solvent B; 40.01–50 min 30–50% solvent B; 50.01–52 min 50–5% solvent B. The flow rate was: 0–5 min 0.1 ml/min; 5.01–15 min 0.2 ml/min; 15.01–35 min 0.1 ml/min; 35.01–50 min 0.2 ml/min; 50–52 min 0.1 ml/min. ESI source and negative ionization mode have been used. The gallic acid, *chlorogenic* acid, ellagic acid, caffeic acid, rutin, rosmarinic acid, luteolin, quercetin, quercetin 3-β-D-glucoside, apigenin, umbelliferone and kaempferol were used as reference standard. Full scan acquisition mode was initially used in m/z range, between 50 and 1000. Further, selected ion monitoring (SIM) mode was used to search for some particular ions.

The identification and quantification of anthocyanidins and peonidin 3-*O*-glucoside have been conducted according to the previously reported HPLC–MS method (Albu Birsan et al., 2017), using a mobile phase water-formic acid (95:5, *v/v*) (solvent A) and methanol-formic acid (95:5, *v/v*) (solvent B). The compounds were

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