International Journal of Biological Macromolecules 79 (2015) 388–391

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Short communication

Chemical and pharmacological profiles of *Echinacea* complex

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ARTICLE INFO

Article history:
Received 28 March 2015
Received in revised form 29 April 2015
Accepted 1 May 2015
Available online 18 May 2015

Keywords:
Echinacea purpurea
Polysaccharide complex
Bronchodilatory effect

ABSTRACT

Echinacea purpurea has a long history in traditional medicine. To verify the pharmacological efficacy of active principles, a polysaccharide–phenolic–protein complex has been isolated from flowering parts of herb by alkaline extraction. It showed on GPC and HPLC one peak of molecular mass around 10 kDa. Chemical and spectroscopic analyses revealed carbohydrate, phenolic and protein contents in *Echinacea* complex. Pharmacological tests have shown its marked cough suppressing and bronchodilatory effects. The antitussive effect of *Echinacea* was similar to the narcotic drug codeine and the bronchodilatory effect was more significant than salbutamol, the antiasthmatic drug used in a clinical practice. Pharmacodynamic study shows the beneficial effects of *Echinacea* complex on the respiratory system and highlights the great potential for development of antitussive and bronchodilatory drugs from natural sources.

1. Introduction

The medicinal plant *E. purpurea* L. (Moench), known as purple coneflower (Asteraceae), is also considered to be an ornamental plant. It has been used in traditional medicine for the treatment of cold, cough, bronchitis, inflammations, etc. [1–5]. Nowadays, it is the most used plant in herbal medicine and in dietary supplements [6]. Recent trends in *Echinacea* are focused mainly on its immunomodulatory effects, particularly in the prevention and treatment of airways infections. The numerous clinical trials were performed to test the efficacy of constituents isolated by different procedures/solvents from various *Echinacea* species to describe their active principles, however, the description of all constituents is still not completed. Generally, four main groups of compounds are considered to be active in *Echinacea* species—alkylamides, phenylpropanoids, glycoproteins and polysaccharides. It seems that these constituents are responsible for the observed immunostimulatory and anti-inflammatory activities [7]. The modulation of immune system was reported by *Echinacea* polysaccharides [8,9]. They increased macrophage chemotaxis, production of reactive oxygen intermediates, enhanced production of TNF-α, IL-10, IL-6 and IL-1-β, assigned adjuvant effects on human T-cell cytokine responses, and inhibited fungi growth [10–12]. Besides, a polysaccharide–phenolic complex was shown to exhibit the anticoagulant activity [13].

Phytoconstituents of *Echinacea* species are still the subject of chemical and pharmacological attention. The aim of this study is to investigate the chemical profile and pharmacodynamic properties of complex isolated from flowering parts of *Echinacea purpurea.*

2. Material and methods

2.1. Plant material, chemicals and isolation of complex

Air-dried flowering parts of *E. purpurea* were purchased from local market and identified by Prof. K.D. Kromer from Botanical Garden of Wrocław, Poland. Citric acid p.a., codeine and salbutamol were purchased from Sigma Aldrich. *Echinacea* complex, salbutamol and codeine were dissolved in water and citric acid in 0.9% saline for application. The isolation of *E. purpurea* complex was made according to described procedure [13]. A dark brown *E. purpurea* complex (**Ep**) was used for chemical and spectroscopic analyses, and pharmacological tests.

2.2. General methods

Carbohydrate, phenolic and protein contents were estimated by the phenol–sulfuric acid, Folin–Ciocalteu and Lowry assays,

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http://dx.doi.org/10.1016/j.ijbiomac.2015.05.010
0141-8130/

respectively [14–16]. Uronic acids were determined by m-hydroxybiphenyl reagent [17]. Samples were hydrolyzed with 2 M TFA for 1 h at 120 °C. The quantitative determination of the neutral sugars was carried out in the form of their alditol acetates [18]. Thin-layer chromatography (TLC) of uronic acids was performed in the system *n*-butanol–ethanol–water (10:8:7). Gel permeation chromatography (GPC) was performed on a column of Sephacryl 100, calibrated with dextran of M_w 2000 and 1.5 kDa. Molecular mass determination of a sample was performed with HPLC Shimadzu apparatus equipped with a differential refractometer RID-6A and a UV–vis detector SPD-10AV using the column HEMA-BIO 1000 (8 mm × 250 mm). A set of dextran standards was used for calibration of the column. Colorimetric assays were measured using UV–vis 1800 spectrophotometer and Fourier-transform infrared (FT-IR) were obtained on a NICOLET Magna 750 spectrometer with DTGS detector and OMNIC 3.2 software, where 128 scans were recorded with 4 cm^{-1} resolution.

2.3. Animals

Adult male Trik strain guinea pigs (200–350 g) were obtained from approved breeding facility and were housed in approved animal holding facility for 1-week adapting period and subsequent several days (2–3) adaptation to experimental conditions. During experiments, a standard air conditioning system and a light/dark cycle with free access to food and water were guaranteed.

2.4. Antitussive and bronchodilatory activity tests in vivo

Animals were placed into a double-chambered bodyplethysmograph and subjected to an aerosol of citric acid (0.3 M) for 3 min to elicit a cough reflex [19]. The total number of cough was recorded during inhalations of this tussigen. The cough response was evaluated prior to vehicle, codeine and *Echinacea* administration (*N* values) and after their application in time intervals 1, 2 and 5 h. Minimal time between two measurements was 2 h to prevent cough receptors adaptation on chemical irritation [20]. The antitussive effect of *Echinacea* was compared to codeine.

The air smooth muscle reactivity was evaluated using the same bodyplethysmograph. The values of specific airway resistance (sRaw) were calculated by Pennock method [21] and their changes were regarded as an indicator of in vivo airways reactivity. The changes in sRaw were measured under the basal conditions and then during 1 min consecutively after the short exposure (30 s) to contractile mediators citric acid (0.3 M). Between the agent exposure and measurement of sRaw was an interval 1 min, during which fresh air was insufflate into the nasal chamber. The effect of *Echinacea* was compared to salbutamol.

2.5. Statistics

Student's *t*-test was used for the statistical analysis of the results. Data are presented as mean ± standard error of the mean (SEM). The results with $p < 0.05$ were considered significant. Significances of $p < 0.05$, $p < 0.01$ and $p < 0.001$ are shown by one, two and three symbols.

3. Results and discussion

3.1. Characterization of Echinacea complex

The alkaline extraction of *Echinacea* flowers afforded a dark brown isolate (**Ep**) in 1.8% yield on dry plant [13]. HPLC analysis showed one single peak of molecule mass at around 10 kDa. GPC confirmed a single-peak profile with high carbohydrate and phenolic components (Fig. 1). Re-examination of **Ep** revealed carbohydrates (26.3%), phenolics (1.03 mM of GAE/1 g, i.e. 17.5%), protein (14%) and uronic acids (11.2%). Carbohydrate analyses showed the dominance of uronic acids (30%), Gal (22%), Ara (17%) and Rha (13%), while other sugars were found in lower contents: Xyl (9%), Glc (6%), Man (2%) and Fuc (1%). TLC analysis of uronic acids confirmed the presence of GalA. Results of sugar analysis suggested the presence of two main polysaccharide components in **Ep**, i.e. rhamnogalacturonan (43%) and arabinogalactan (39%).

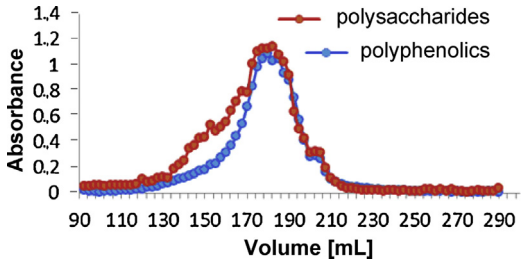

Fig. 1. GPC of *Echinacea* complex on Sephacryl S-100 column.

To confirm the structural components in **Ep**, it was analyzed by FT-IR spectroscopy (Fig. 2). The bands found at 1593 and 1404 cm^{-1} derive from the antisymmetric and symmetric vibration mode of carboxyl groups (COO^-) of uronic acids, respectively, and indicate thus the presence of acidic polymers in **Ep**. Bands characteristic for the presence of polysaccharide moieties found at 1145, 1069 and 1043 cm^{-1} correspond to stretching vibrations of ν(C—OH), ν(C—O—C) and ν(C—C) bonds, and ring vibrations [22]. The low shoulder found at around 1516 cm^{-1} is due to double bounds in aromatic rings and the bands in the region 1324–1247 cm^{-1} indicate stretching and bending resonances of C—O—H groups derivable from carboxyl groups of phenolics [23]. The presence of protein in **Ep** could be recognized by small shoulders at 1650 (amide I) and 1545 cm^{-1} (amide II) arising from stretching vibrations of ν(C=O) and bending vibrations of δ(N—H) bonds, respectively [23]. But, bands due to protein were fused with strong carboxyl band $\nu(COO^-)$ at 1599 cm^{-1}, derivable from uronic acids.

To verify the presence of protein in **Ep**, uronic acids were converted into protonized form (**EpH**$^+$) and thus providing only one band for carboxyl groups in FT-IR spectrum. The bands due to protein can be more visible in the region 1655–1545 cm^{-1}. The FT-IR spectrum of **EpH**$^+$ revealed changes in the region of 1800–850 cm^{-1} (Fig. 2). In the spectrum appeared new bands at 1716, 1655, 1640, 1545, 1522, 1221 and 893 cm^{-1}. The band at 1716 cm^{-1} derives from ν(C=O) resonances of COOH groups of uronic acids. The relatively intense bands at 1640, 1522 and 1221 cm^{-1} derive from phenolics, however, the band at 1640 cm^{-1} includes the water vibration as well, but its intensity is usually low. The low intensity band at 893 cm^{-1} is due to the β-anomeric configuration of galactose units, which are part of the arabinogalactan, one of the main polysaccharide components of Ep. The band at 1655 (amide I) and the low shoulder at 1545 cm^{-1} (amide II) are due to protein content. The FT-IR measurements confirmed the presence of three structural components in **Ep** including carbohydrates, phenolics and protein.

3.2. Bronchodilatory and antitussive effects of Echinacea

Effects of *Echinacea* complex on the airways smooth muscle reactivity and the cough reflex were examined in vivo using the bodyplethysmograph. The specific airway resistance (sRaw) is a parameter used in respiratory physiology to describe the mechanical factors, which limits the access of inspired air to the lower parts of the airways and thus, determines airflow. It is strongly related to the diameter of the airways. Pharmacological tests showed