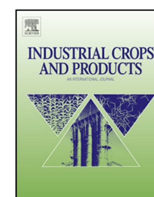
Industrial Crops and Products 86 (2016) 68–72

Contents lists available at ScienceDirect

Industrial Crops and Products

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



Short communication

Impact of harvest time of *Aesculus hippocastanum* seeds on the composition, antioxidant capacity and total phenolic content

Bartosz Kędzierski[a], Wirginia Kukula-Koch[a,*], Jarosław Widelski[a], Kazimierz Głowniak[a,b]

[a] Chair and Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1, Chodzki str., 20-093 Lublin, Poland
[b] Department of Cosmetology, University of Information Technology and Management in Rzeszów, 386a, Kielnarowa, 36-020 Tyczyn, Poland

ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form 18 January 2016
Accepted 11 March 2016
Available online 24 March 2016

Keywords:
Horse chestnut
Antioxidant activity
LC–MS analysis
Phenolics
Harvest time
Escin

ABSTRACT

Horse chestnut fruits and seeds have been traditionally used as a remedy against chronic venous insufficiency symptoms. Currently the total extracts or purified saponins are extensively used as dietary supplements or medicines' ingredients in numerous authorized pharmaceuticals. The goal of this study was to investigate the changes in the secondary metabolites content within time. The selected optimal conditions for collection could provide the highest quantity of active compounds in the extracts, which will increase the quality of the final product. The obtained results indicate that the highest content of natural products in the horse chestnut fruits was measured between the 13th and 16th week from the beginning of blooming. The quantitative analysis of escin and two flavonoids: kaempferol and procyanidin A2 was performed on the extracts within the study. The content of the selected natural products increased of ca. 90% between the 12th and 20th week for escin and of ca. 120% between the 7th and 12th week, for flavonoids. The rise in natural products quantity was also expressed in the measured antioxidant activity and total phenolic content values in DPPH and Folin–Ciocalteu tests.
© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Horse chestnut, *Aesculus hippocastanum* L. (Hippocastanaceae), is a large, deciduous tree originating from Balkans. Its fruit and seed extracts have been traditionally used in the treatment of chronic venous insufficiency, usually manifested by varicose veins, hemorrhoids, swelling, pain and heaviness of lower limbs (Zhang et al., 2010) due to the presence of escin, characterized by three types of pharmacologic properties: anti-oedematous, anti-inflammatory and venotonic activities (Matsuda et al., 1997; Sirtori, 2001). Escin saponins (see Fig. S1) contain two major groups of isomers with different physicochemical properties and pharmacological activities: β-escins (more active ones), containing escin 1a and escin 1b, and α-escins (composed of isoescin 1a, isoescin 1b). Among other constituents of the extracts sterols, flavonoids (EMA, 2009) – kaempferol and quercetin glucosides, epicatechin and its dimer procyanidin A2 were described (Fig. S2) (Chen et al., 2007; Wilkinson and Brown, 1999).

According to the literature, significant differences in the composition of extracts obtained from both different organs of the plant (bark, fruits, seeds), but also within a vegetation period were observed (Dudek-Makuch and Matławska, 2013). The content of coumarins, such as esculin or fraxin in the chestnut was evaluated by Stanic et al. (1999) after the collection of its bark in four following seasons and revealed marked differences between the samples, with the highest coumarin content in the bark gathered from older branch sections.

However, to the best of our knowledge, no data has been presented on the variation of the pharmacologically most important compound, present in various products, dietary supplements and medicines, namely escin. In our study we evaluated also kaempferol and procyanidin A2 contents in the extracts in the plant maturation process. Chestnut's flavonoids, which are represented by kaempferol, constitute an important group of secondary metabolites in the seeds, and are supporting the overall action of chestnut, causing the spasmolytic effect on veins (Kukula-Koch et al., 2014).

Additionally, the antioxidant capacity and the TPC of the seeds collected at different times were investigated to observe the compositional changes in the plant.

Abbreviations: ASE, accelerated solvent extraction; TPC, total phenolic content; FA, formic acid.

* Corresponding author.
E-mail addresses: bartosz.kedzierski1@gmail.com (B. Kędzierski), virginia.kukula@gmail.com (W. Kukula-Koch), jwidelski@pharmacognosy.org (J. Widelski), kglowniak@pharmacognosy.org (K. Głowniak).

http://dx.doi.org/10.1016/j.indcrop.2016.03.034
0926-6690/© 2016 Elsevier B.V. All rights reserved.

It is worth mentioning, that currently escin is obtained on an industrial scale by various isolation techniques from natural resources—from horse chestnut fruits or seeds. The estimation of escin content in various periods of horse chestnut vegetation is significant because of high utilization of this active compound in pharmaceutical industry for medicinal purposes and dietary supplementation. The herein presented results optimize the collection of chestnut fruits or seeds to obtain an enriched raw material of an enhanced pharmacological activity.

2. Materials and methods

2.1. Plant material

The samples ripening fruits, 10 pieces each time, were collected at different vegetation periods from four trees growing in Poland (Warsaw area—1 tree, Lublin area—3 Trees—from a city park, forest and from the outskirts of the city), on the following weeks (calculated from the start of flowering): 7th week, 10th week, 12th week, 14th week, 16th week, 19th week, 21th week (samples appearance is presented in Fig. S3). The seeds from the first tree (Warsaw area) were crushed and dried at a temperature of 35 °C for a day and stored in a dry, cool and dark place until further extraction. The remaining ones were deep frozen at a temperature of −25 °C and were defrozen, crushed and dried as previously before the analysis. The voucher specimen of each sample is stored in the Chair and Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Poland under a number (BK2014001-BK2014007, BK22014001-BK22014007, BK32014001-BK32014007, and BK42014001-BK42014007).

2.2. Chemical reagents

All chemicals used in the extraction process, TPC assay an radical scavenging assay (excluding DPPH free radical) were purchased from Avantor Performance Materials, Poland in Gliwice. The chemicals suitable for the HPLC and LC–MS analysis (including MeOH, ACN, H_2O, FA of spectroscopic purity) were purchased from J.T. Baker. The standard of escin (purity≥95%), rutin (purity≥95%) and DPPH radical were purchased from Sigma Aldrich (St. Louis, USA).

2.3. Sample preparation and extraction

4 g of each dried sample was weighted and extracted by accelerated solvent extraction with MeOH (ASE 100, Dionex, USA). The following extraction conditions were applied: extraction temperature: 90 °C, flush volume: 80%, purge time: 100 s, number of cycles: 2, duration of a cycle: 10 min. The extracts were evaporated under reduced pressure at 50 °C. Dried residues were weighed (see Fig. 1B). 10 mg of each dry extract were dissolved in 1 mL of MeOH and subjected to HPLC and LC–MS analysis.

2.4. HPLC analysis

The analysis was performed on Shimadzu HPLC apparatus equipped in an autosampler (SIL-20 A HT), a degasser (DGU-20 A), a quaternary pump (LC-20 CE), a DAD dectector (SPD-M 20A), a thermostat (CTO-10 AS VP), and a C8 column (Hypersil BDS 5 μm C8 250 mm × 4.6 mm). In order to achieve sufficient resolution of the peaks, a suitable method was developed for this study. 0.1% solution of FA and ACN with addition of 0.1% of FA were applied as solvent A and B, respectively. Mobile phase flow was set at 1 mL/min, postrun at 4 min and a specific gradient of solvents was used. The following mobile phase gradient was applied in the chromatographic separation: 0 min—1% B in A, 0–10 min—1–8% B in A, 10–20 min—8–10% B in A, 20–50 min—10–20% B in A, 50–70 min—20–40% B in A, 70–100 min—40–60% B in A, 100–105 min—60–75% B in A, 105–110 min—75–1% B in A. The detection was carried out at 254 nm, at 25 °C. The calibration curve of ß-escin was prepared from 6 dilutions of the 0.5 mg/mL solution giving the R^2 value of 0.9995 and the following calibration curve equation $Y = 223391x + 415.06$ used for the quantitative analysis (see Fig. S9).

2.5. LC–MS analysis

The LC-ESI-TOF-MS apparatus was operated in the positive mode, according to the method previously published by the authors (Kukula-Koch et al., 2014), to confirm the identification of compounds after HPLC analysis.

2.6. DPPH radical scavenging assay and total phenolic content measurement

Both assays were performed using a previously elaborated method published by the authors (Kukula-Koch et al., 2014). The concentration of DPPH methanolic solution and horse chestnut extracts solution in DMSO was 5 mg/100 mL and 10 mg/mL, respectively. Standard calibration curve of gallic acid was prepared (see Fig. S4A). The results of Folin–Ciocalteu assay were expressed as gallic acid equivalents, based on the standard calibration curve of gallic acid (see Fig. S4B). All plant extracts in TPC assessment were dissolved in DMSO to a concentration of 5 mg/mL prior to testing.

2.7. Statistical analysis of data

The statistical analysis of the obtained data was performed in the Statistica (10.0) program. For the fruits of each tree, 5 separate extractions were performed and the results were expressed as average values ± standard deviations. The significance of the results was determined at $p < 0.05$.

3. Results and discussion

3.1. Extraction, quantitative and qualitative analysis of the extract

Extraction conditions are important factors influencing the efficiency of natural products recovery. The conditions applied were optimized by the authors in the preceding studies (Kukula-Koch et al., 2014). Seven extracts obtained by ASE from the same amount of raw material collected in different harvest times of the same season, were analyzed. Some significant differences in the obtained dried residue weight and in the composition of the extracts have been observed (see Fig. 1A–D). The extraction yield increased within the maturing process from the 7th until the 14th week, and decreased gradually in the following weeks. The extracts obtained in the 14th week have shown the highest quantity of dried residue (see Fig. 1B).

Thorough qualitative HPLC and MS-based analyses of all extracts were performed to deliver a simple, fast and effective separation of enriched extracts (see Fig. S6, S8). Several method parameters were optimized: the gradient profile, composition of a buffer, flow rate and the injection volumes. Positive mode was found preferable for the analysis of horse chestnut's extracts, giving more efficient ionization and less crowded spectra. The LC–MS method was developed at first with mobile phase consisted of water with the addition of 0.1% FA and a mixture of MeOH and ACN (50:50 V/V) with 0.1% of FA. Optimization trials were performed to fit specific gradient of solvents in order to obtain good separation of peaks, however, the presence of methanol disturbed the detection of saponins in the applied wavelength (210 nm and 254 nm). That is why MeOH was removed, what improved the clarity of peaks. In further trials the

Download English Version:

https://daneshyari.com/en/article/4512519

Download Persian Version:

https://daneshyari.com/article/4512519

Daneshyari.com