**Introduction**

Aesculus hippocastanum L. Hippocastanaceae is spread in the northern hemisphere, primarily in eastern Europe, eastern Asia and eastern North America. Plant material of medical interest is dried ripe seeds. The principal ingredient of Horse Chestnut seed is escin, which has an anti-exudative vascular tightening effect and reduce vascular permeability which provokes an antiedemic effect. The vein-toning properties of the Horse Chestnut seed extract also demonstrated improvement of venous return flow. Horse chestnut seed extracts are indicated for treatment of complaints of chronic venous insufficiency such as feeling of pain, heaviness, and tension in the legs, nocturnal swelling of the legs and pruritis. Escin inhibits the activity of lysozymal hyaluronidase by preventing break-down of the main component hyaluronic acid, and thereby preserving the matrix that supports the capillary walls.

**Aim of study**

The aim of the presented study was optimization of the extraction conditions of escin from, not the whole seeds, but from dried horse chestnut seeds crust. Also, the effects of extractions parameters such as the extraction temperature, flow rate and the extraction time were investigated.

**Experimental part**

**Materials and reagents**

The seeds were collected in gardens at Vrnjačka Banja in Serbia. All the reagents such as methanol, ethanol, chloroform and acetic acid of analytical grade and were obtained from local chemical suppliers. Standard escin was obtained from Santa Cruz Biotechnology California.

**Sample preparation**

The horse chestnut seeds were dried at room temperature and ground into powder with a cyclone mill and passed through a number 1400 sieve. The hippocastani seed powder was treated with 67 % v/v of ethanol. The process of extraction was carried out in a laboratory by the extractor Big Max 5. Šamtech, Austria. The ratio of drug to the extract was 2.5. Photometric determination of triterpene glycosides (calculated as total escin amounts) is based on the color reaction of the products from the reaction with mixture of acetic acid, sulfuric acid and conc.ferry chloride (DAB X).

**Experimental design and statistical analysis**

This study was aimed to optimize the extraction conditions of escin from dried horse chestnut seeds crust investigating the effects of extractions parameters such as the extraction temperature, flow rate and the extraction time. The content of the escin was analyzed using pharmaceupotia method (DAB, 1991). In the German Pharmaceupia the content of escin of Aesculus hippocastanum and its extract are determined by means of a colorimetric method. This method provides determination of only the total saponins content and is non-specific towards individual escin (the saponins escin is a natural mixture of triterpene saponins).

A three-variable, two-level full factorial design (FFD) was applied in order to evaluate the influence of critical factors on process of extraction and in order to obtain maximum extraction yield in examined experimental domain. On the basis of preliminary experiments three factors were chosen as independent variables: extraction temperature (°C), flow rate of solvent (ml min⁻¹) and extraction time (h). Factors and their “low” (−1), “high” (+1) and “zero” (0) values are presented in Table 1.

**Conclusion**

The investigation was carried out in two steps. The objective of the first step was to perform a screening of the factors that could potentially influence to process of extraction and the second was employing the multicriteria methodology in order to get the best operating conditions. Since the number of influencing factors is three and since it does not require an excessive number of experimental runs, full factorial design (FFD) was chosen. A total of 11 experiments were designed. Each experiment were performed in triplicate and average extraction yield (Ey) was chosen as the response, Y. Experimental layout designed by Design-Expert and corresponding experimental values of responses is presented in Table 2.