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METODA SIMPLĂ ȘI ECOLOGICĂ DE IZOLARE A ROBININEI DIN FLORI DE SALCÂM ALB *(ROBINIA PSEUDOACACIA L.)*

A SIMPLE AND ECOLOGICAL METHOD FOR ROBININ ISOLATION FROM BLACK LOCUST FLOWERS *(ROBINIA PSEUDOACACIA L.)*

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Abstract. Significant reserves of black locust on the territory of the Republic of Moldova make this species promising for the production of robinin, the main glycoside of kaempferol, contained in flowers and having hypoazotemic, diuretic and other pharmacological properties. However, known methods of isolation require the use of harmful organic solvents. This work aimed to create a simple and ecological method for isolating robinin from black locust flowers. The problem was solved by using a variation of the solvent replacement method, in which ethanol is removed from the primary hydroalcoholic extract by evaporation, resulting in the crystallisation of robinin. Its following purification was carried out by recrystallisation from water with simultaneous sorption of impurities by activated carbon. The target product is obtained in the form of a light yellow crystalline powder with a main substance content of at least 85% and a yield of about 60% of the content in plant material.

Keywords: Robinia pseudoacacia L., robinin, isolation, solvent replacement method.

Rezumat. Rezervele semnificative de salcâm alb pe teritoriul Republicii Moldova fac ca această specie să fie promițătoare pentru producția de robinină, principala glicozidă a kaempferolului, conținută în flori și având proprietăți hipoazotemice, diuretice și alte. Totodată, metodele cunoscute de izolare a acesteia necesită utilizarea solvenților organici nocivi. Scopul acestei lucrări a fost de a crea o metodă simplă și ecologică pentru izolarea robininei din flori de salcâm alb. Problema a fost soluționată prin utilizarea unei variante a metodei de înlocuire a solventului, în care etanolul este înlăturat din extractul hidroalcoolic primar prin evaporare, rezultând cristalizarea robininei. Purificarea sa ulterioară s-a realizat prin recristalizare din apă cu sorbția simultană a impurităților de către cărbunele activat. Produsul țintă se obține sub formă de pulbere cristalină galben deschis, cu conținutul substației de bază de cel puțin 85% și un randament de aproximativ 60% din conținutul în materia primă vegetală.

Cuvinte cheie: Robinia pseudoacacia L., robinină, izolarea, metoda de înlocuire a solventului.

INTRODUCTION

Black locust (*Robinia pseudoacacia* L.) is a widespread plant of the *Fabaceae* family. It was first introduced in Europe in 1601, and in Romania in 1750 as an ornamental tree, and from the middle of the 18th century spread over large areas due to its unpretentiousness to weather and climatic conditions and soil composition. Currently, it occupies about a third of the entire forest area of the Republic of Moldova [1]. Black locust is one of the most valuable honey plants, produces high-quality wood and effectively prevents soil from landslides, which determines its high economic value [1,2]. At the same time, the invasiveness of this species creates some environmental problems [2]. Black locust is still not used in official medicine, but it is known that the inflorescences contain flavonoids, in particular kaempferol glycosides, the main of which is robinin (kaempferol-3-O-robinoside-7-O-rhamnoside) [3]. Robinin has a diuretic and hypoazotemic effect and was used in medical practice in the form of the commercial drug „Flaronin", obtained from *Astragalus falcatus* Lam. and intended for the treatment of chronic renal failure [3-5]. Robinin was described also to have antidiabetic properties, related to its ability to inhibit dipeptidyl peptidase IV [6], and anti-inflammatory activity [7]. Significant reserves of black locust on the territory of the Republic of Moldova are of interest as a raw material base for the robinin production. However, the described methods for its isolating from this plant species require the use of unsafe solvents, such as acetone, ethyl acetate, and butanol [8], or pyridine [9], which is not attractive from both economic and environmental points of view.

The objective. Creation of a simple, inexpensive and environmentally safe method for isolating robinin from black locust flowers for the purpose of its following study as a pharmaceutical substance.

MATERIAL AND METHODS

Black locust inflorescences were collected at the end of budding - the beginning of flowering from forest plantations in the central region of the Republic of Moldova and dried under natural conditions.

Isolation of robinin from plant material was carried out as follows: 0.65 kg of black locust flowers were percolated with 60% ethanol at speed of 200 ml/h and room temperature to obtain 3.25 L of primary extract (1:5). Ethanol was distilled from the filtered extract at atmospheric pressure till vapour temperature of 97°C. The hot residue was transferred into a separatory funnel, allowed to cool slowly to 50°C, then cooled with flowing water to room temperature. The separated resin was drained carefully from the bottom tap of the funnel. The remaining liquid was centrifuged and filtered through cheesecloth. To the resulting concentrated extract (approximately 1.0 L), 1 ml of benzyl alcohol was added as a preservative and left to stand for 3 weeks at room temperature for the crystallisation of robinin. The main part of the liquid phase was decanted, the rest was centrifuged. The precipitate was washed three times with water, in portions of 0.15 l, by suspension followed by centrifugation. Then the precipitate was suspended in 0.85 l of water, heated with stirring until the main part dissolved. 1.7 g of activated carbon was added, heating and stirring continued until boiling, then liquid was immediately filtered under weak vacuum through 2 layers of filter paper on a Buchner funnel. The filtrate was transferred to a conical flask, heated until the formed insignificant precipitate dissolved and allowed to cool slowly until the next day. The crystalline precipitate was separated on a large-pore glass filter, washed with small amount of water and dried at 40°C. 6.6 g of the target product was obtained.

Analysis of plant material, extracts, and final product has been performed using *Agilent 1260* liquid chromatograph with diode-array detector in following conditions: Analytical column Zorbax Eclipse XDB-C8, 5 mkm, 4.6 x 150 mm; linear gradient from 4% to 40% acetonitril in 0.05% trifluoracetic acid at 1.5 ml/min; detection at 350 nm with online spectra registration. As a working standard, we used robinin, isolated as described above, purified additionally by triple recrystallisation from 96% ethanol, and standardised by primary analytical standard from *Symit Quimica S.L.* (Spain). The reference substances robinin, rutoside, and apigenin-7-glucoside were used to identify corresponding aglycones in the composition of flavonosides, found on chromatograms, by similarity of its UV spectra.

RESULTS AND DISCUSSIONS

The robinin content in the used plant materials, determined by HPLC, was 1.7% by weight. The proposed method for isolating robinin is based on a variant of the solvent replacement method, in which one of the two solvents is removed from the mixture by evaporation. At the same time, the solution of the target component becomes concentrated, which also contributes to its release into the dispersed phase.

The optimal concentration of ethanol in the extragent was 60%. Lower concentrations give a reduced yield of robinin, and higher concentrations lead to the extraction from the raw material of significant amounts of resinous substances, which, tending to float, complicate the processing of the concentrated extract. If the ethanol concentration in the extragent is not more than 60%, resinous substances sediment from concentrated extract to the bottom of the vessel, which is facilitated by slow cooling of the extract to 50°C. Lower temperatures create conditions for crystallisation of robinin, so further cooling was carried out quickly, followed by immediate separation of the resin and centrifugation of the aqueous phase. Visible crystallisation of robinin begins after 1-2 hours at room temperature, but it takes at least 2-3 weeks to complete, due to the increased viscosity of the extract and the high concentration of easily soluble sugars. On the other hand, slow crystallisation promotes the formation of fairly large crystals, which has a beneficial effect on the purity of the obtained product.

The crude robinin purification was carried out by recrystallisation from hot water with simultaneous sorption of slightly polar impurities with activated carbon. The yield of purified robinin is about 60% of its content in the plant material. The final product is a light yellow crystalline powder. It contains at least 85% robinin and about 3% water. The main impurities are related glycosides of kaempferol, which presumably have similar pharmacological properties (Figure 1).

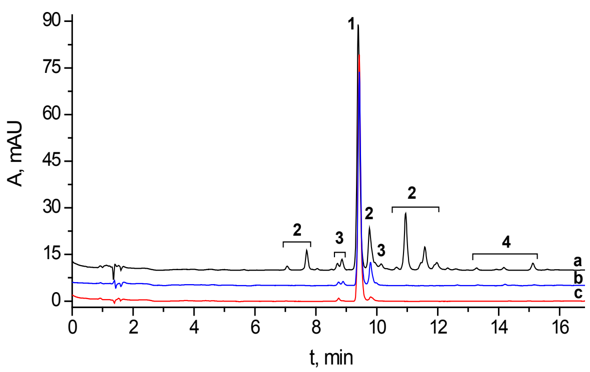

Figure 1. Chromatograms of black locust extract (a), isolated robinin (b), and working standard (c): 1 – robinin; 2 – other kaempferol glycosides; 3 – quercetin glycosides; 4 – apigenin glycosides.

CONCLUSIONS

A simple, inexpensive and ecological method for isolating robinin from black locust flowers is proposed. The method is based on the crystallisation of robinin from a concentrated aqueous solution obtained by evaporating ethanol from the primary hydroalcoholic extract, and following purification of the crude product by recrystallisation from water with simultaneous treatment with activated carbon.

The final product contains at least 85% of the main substance and can be studied as a potential pharmaceutical substance.

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