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Research Article

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Identification and Determination of Quantitative Content of Flavonoids in Desmodium Canadense (L) DC Herb of Persei Variety

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ABSTRACT

Homoorientin and saponaretin were identified in Desmodium canadense herb of Persei variety by the TLC method. A quantitative content of flavonoids homoorientin and saponaretin was established by the spectrophotometric method in terms of dry raw material.

Keywords: Desmodium; Flavonoids; TLC; Spectrophotometric

INTRODUCTION

Desmodium (*Desmodium canadense* (L.) DC., Fam. *Fabaceae*) - is a perennial herbaceous plant that occurs in North America. This plant in many countries, including Ukraine, is cultivated [1].
The Desmodium canadense herb contains flavonoids such as apigenin, apigenin-7-O-glucoside, luteolin, rutin, 2-vicenin, vitexin, isovitexin, vitexin rhamnoside, orientin, homoorientin, quercetin, hyperoside, astragalin, kaempherol [2,3]. In addition, it also contains saponins and phenolic acids (chlorogenic acid, vanillic, 4-hydroxycinnamic, ferulic and caffeic) [1,4]. Desmodium herb exhibits antioxidant, antibacterial, anti-inflammatory, hepatoprotective, diuretic, analgesic activity [5]. It is known that C-glycosides of flavonoids exhibit antioxidant, hepatoprotective, anti-inflammatory and antiviral effect [6].
Therefore the identification of flavonoids, namely C-glycosides, and determination of their quantitative content in the Desmodium canadense herb of Persei variety is relevant. The purpose of the work was the identification and quantification of flavonoids in the Desmodium canadense herb of Persei variety.

EXPERIMENTAL SECTION

Identification of flavonoids in the Desmodium canadense herb of Persei variety was perfomed by TLC, determination of quantitative content – by the spectrophotometric method [7].

Identification

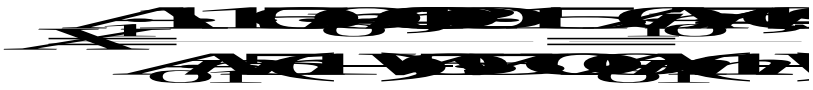
10 µl of solution A (prepared as described in quantitative measurement method), 10 µl of solution of the rutin reference substances (RS) (2.5 µg) and hyperoside (2.5 µg), 5 µl of rutin RS solution (1.25 µg) and hyperoside (1.25 µg) were applied on the spotting line of the chromatographic plate "Kieselgel 60 F_{254}" (Merck) with size 10 x 20 cm.
The plate with the applied specimens were air dried for 10 min, placed into the chamber with the mixture of such solvents as butanol–glacial acetic acid–water (30:5:10) and derived by ascending technique. When the solvent front passed about 15 cm from the spotting line, the plate was taken out of the chamber, air-flow dried for 30 min, sprayed with 4% aluminum chloride solution and dried in drying chamber at 100°C to 105°C for 3 min. The plate was looked through under the ultraviolet light at 366 nm.

Preparation of rutin and hyperoside RS solutions

0,025 g of rutin and 0,025 g of hyperoside were put into 100 ml volumetric flask, dissolved in 60 ml of 70% ethanol while heating on the water bath, cooled up to the ambient temperature, made the solution volume up with the same solvent to the mark and mixed.

Preparation of 4% aluminum chloride solution
4 g of aluminum chloride were put into 100 ml volumetric flask, dissolved in 60 ml of 70% ethanol, made the solution volume up with the same solvent to the mark and mixed.

Quantitative measurement
About 2,5 g (accurately weighed) of the powdered raw material were put into a round-bottom 250 ml flask, where 50 ml of chloroform was added and heated with the back flow condenser within 10 min by keeping moderate solution boiling. Then this was cooled, filtered through the cotton and the chloroform phase was further removed. The procedure was repeated twice according to the method starting from the words "…50 ml of chloroform was added…" by filtering through the same cotton. This cotton was put into the same round-bottom flask after filtration. The chloroform remnants were reduced on the boiling water bath, added 25 ml of 70% ethanol into the flask and heated with the back flow condenser on the hot water bath with reflux condenser within 10 min. Then this was cooled to the ambient temperature and decanted through the "blue ribbon" filter into 100 ml volumetric flask. The residue in the flask was extracted 3 more times according to the same method, starting from the words "…25 ml of 70% ethanol was added …" by decanting the solution into the same volumetric flask through the same filter. The solution volume was made up with 70% ethanol to the mark and mixed (solution A).
5 ml of solution A were put into the 50 ml flask, where 1 ml of 4% aluminum chloride solution was added, then in 10 min 5 ml of buffer solution with pH=4,2 was added, the solution volume was made up with 70% ethanol and mixed (solution B).
Optical density of solution B was measured with spectrophotometer at 384 nm in a cuvette with the thickness layer of 10 mm, using as the reference solution the solution consisting of 5 ml of solution A. 5 ml of the buffer solution with pH=4.2 was placed into 50 ml volumetric flask and made up with 70% ethanol to the mark, and then mixed.
In parallel the optical solution density of a solution, consisting of 4 ml rutin RS solution, 8 ml of 4% aluminum chloride solution, 5 ml of buffer solution with pH=3,3, which were placed into the 50 ml volumetric flask and made up with 70% of ethanol and then mixed, was measured.
Content of sum of flavonoids homoorientin and saponaretin *(X)* in terms of rutin and absolutely dry matter in % was calculated by formula as follows:



A_1 – optical density of the solution B;
A_0 – optical density of rutin RS;
m_1 – weight of the raw material sample, g;
m_0 – weight of the rutin RS sample, g;
W – Moisture content in the raw material, %;
0.2579 – ratio of the rutin specific absorption at 384 nm to specific absorption of homoorientin and saponaretin at the same wavelength

Preparation of rutin RS solution
0.05 g (accurately weighed) of rutin having been previously dried at temperature from 130°C to 135°C for 3 hours were put into 50 ml volumetric flask, dissolved in 30 ml of 96% alcohol while heating up on the warm water bath, cooled to the room temperature, made the solution volume up with the same solution and mixed.

Preparation of buffer solution pH=4.2
10 ml of 1 M sodium hydroxide solution were put into 100 ml volumetric flask, 39.6 ml of 1 M acetic acid solution were added, made the solution volume with water to the mark and mixed.

Preparation of buffer solution pH=3.3
4 ml of 1M sodium hydroxide solution were put into 100 ml volumetric flask, the volume solution with 1 M of acetic acid made to the mark and mixed.

RESULTS AND DISCUSSION

As a result of the studies performed the two main spots have been discovered and identified as follows: the first spot was at the level of rutin spot on rutin RS chromatogram and hyperoside R_f about 0.4 (saponaretin), the second spot: at the level of hyperoside spot on rutin RS and hyperoside R_f about 0,6 (homoorientin).

CONCLUSION

In the Desmodium canadense herb of Persei variety by the TLC method flavonoids homoorientin and saponaretin were identified. The quantitative content of flavonoids has been determined by the spectrophotometeric method. The results of the studies will be used in the standardization of Desmodium canadense herb of Persei variety.

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