

Determination of protodioscin in *tribulus terrestris* by reversed-phase high-performance liquid chromatography

Mikhail Sergeev¹, Georgiy Zaitsev², and Dmitry Yermolin^{1,*}

¹V.I. Vernadskiy Crimean Federal University, Prospekt Vernadskogo 4, Simferopol, Republic of Crimea, Russia

²Magarach All-Russia National Research Institute for Viticulture and Wine-Making, st. Kirov 31, Yalta, Republic of Crimea, Russia

Abstract. In this study, high performance liquid chromatography (HPLC) was used to analyze the protodioscin content in *Tribulus terrestris* samples. They were separated by C18 chromatography column, gradient with 0.1 % phosphoric acid solution and acetonitrile was used as the mobile phase with the flow rate of 0.5 mL/min, the column temperature was 45 °C, and the detection wavelength was 203 nm. The results showed that the protodioscin content has a good linear correlation ($r > 0.999$) within the range of 50 mg/L to 1500 mg/L. In samples of raw material from Crimea it was found: $0.546 \% \pm 0.013$ protodioscin and in analyzed material from Western Siberia it was $0.338\% \pm 0.008$ for $n=3$. Raw materials of *Tribulus terrestris* growing in Crimea are more promising than similar raw materials from the West Siberian region due to the higher content of protodioscin for the production of biologically active products containing steroid saponins.

1 Introduction

Tribulus terrestris is an annual plant in the caltrop family (Zygophyllaceae). It grows in South Asia, Africa, Europe, northern Australia, Russian Federation (Crimean Republic, North Caucasus, Western and Eastern Siberia). *Tribulus terrestris* infusions, decoctions are widely used in alternative medicine in many countries. Bulgaria - erectile dysfunction treatment, Iran as a diuretic, laxative and syphilis treatment, India - radiculitis, in traditional Chinese medicine - eye diseases treatment [1]. In science-based medicine *Tribulus terrestris* extract is used as a booster for reproductive system. It is based on significant concentration of furostanol-type steroidal saponins in the plant represented by dioscin, protodioscin, tribestin, prototribestin, methylprototribestin, methylprotodioscin, pseudoprotodioscin, tribulosin, tribulusamide C and other compounds [2]. High concentrations of flavonoids, amino acids, lipids are also represented in *Tribulus terrestris* [3-6].

Protodioscin is a highliter to *Tribulus terrestris* extract quality control [7-9]. Preparations, containing furostanol glycosides are used to cardiovascular disease treatment [10].

*Corresponding author: dimayermolin@mail.ru

On Figure 1, the biologically active compounds in *Tribulus terrestris* chemical structure is presented.

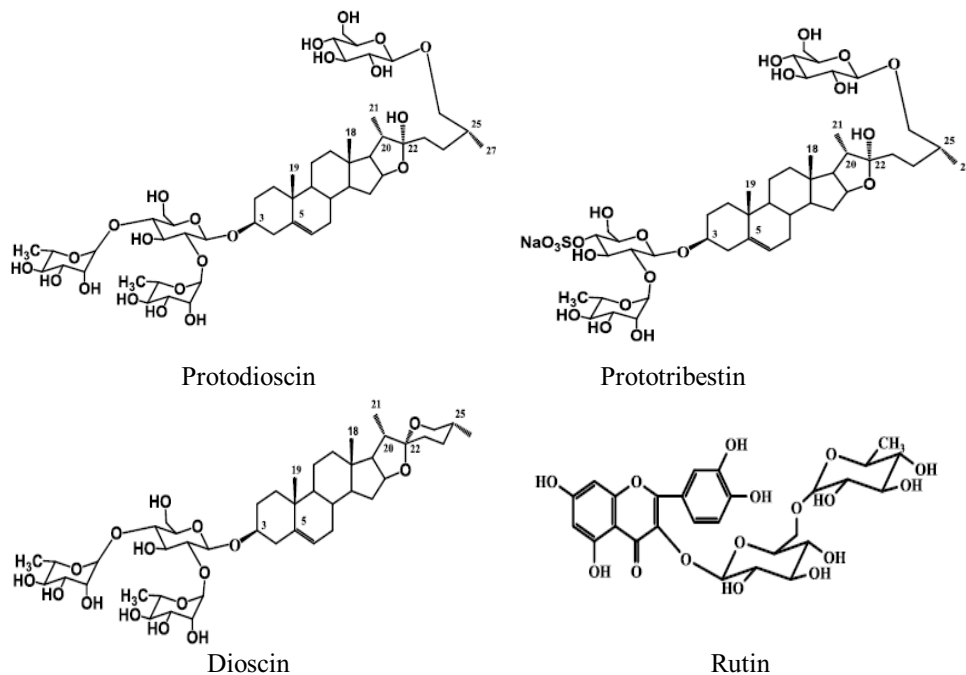


Fig. 1. Structural formulae of the biologically active compounds in *Tribulus terrestris*.

Nutritional supplements with *Tribulus terrestris* extract are widespread among people involved in physical education and sport [11]. Plants bioactive substances helps to rise testosterone production. It is used as testosterone booster

Saponins bind to the hypothalamus receptors. The hypothalamus signals the start of luteinizing hormone production [12].

Studies were made by panel scientists headed by Kazys Milasius. It showed that *Tribulus terrestris* supplements usage significantly helps to double or triple testosterone level due to the 8-20 days course of administration [13]. The same study found that *Tribulus terrestris* consumption result certain increase of strength and aerobics activities data within sportsmen.

There are sources that illustrating the need for additional research on the *Tribulus terrestris* bioactive substances impact upon sportsmen physical efficiency [14].

S.G. Angelova, Z. Gospodinova, M. Krasteva и G.G. Antov illustrates *Tribulus terrestris* cancer protection abilities. It was set that general bulgarian *Tribulus terrestris* extract has a pronounced dose-related and abscopal effect on breast cancellation viability. Saponin fraction has an increased inhibitory effect compared to the total extract. Apoptotic bodies are involved in *Tribulus terrestris* anticancer activity mechanisms. Structural changes and DNA fragmentation were observed as markers of early and late apoptosis in tumor cells after treatment [15].

The study is intended to define protodioscin in *Tribulus terrestris* through reversed-phase high-performance liquid chromatography.

2 Materials and methods

2.1 Materials

Plant material. The study of the protodioscin content have been carried out with a plants, natively distributed in Crimea and Western Siberia. The aerial parts plants (leave, stem, fruit) were collected in September 2020 year.

Protodioscin standard was acquired in Sigma-Aldrich (G0299, CAS 55056-80-9). Acetonitrile was of HPLC grade and purchased from Lab-Scan (Poland).

2.2 Sample Preparation

Exactly 1.2 gram of the finely powdered plant material was extracted with 10 mL of 90% aqueous methanol by sonication within 60 min. After one day of infusion, the extracts were centrifugation and filtered through a 0.2- μ m Iso-Disk Nylon filter (Supelco). Every sample solution was injected in triplicate.

2.3 Calibration

Standard compounds protodioscin (3 mg) was dissolved in 2.00 mL of 50% aqueous methanol (stock solution); further calibration levels were prepared by diluting the stock solution with the same water/methanol mixture. The resulting range for the concentration of protodioscin was from 50 to 1500.0 mg/L. All calibration levels were injected in triplicate, and the calibration data obtained ($R^2=0.9999$ and $y=1.379X+5.34$) indicated linearity of the detector response in the range already mentioned.

Analytical Method. HPLC analysis was performed on a Agilent 1260 Infinity II LC system (equipped with a quaternary pump VL (G7111A), a vialsampler (G7129A), a multicolumn thermostat (G7116A), and a diode array detector VL (G1315D) from Agilent Technologies, Inc., Germany. A UCT Selectra AQUEOUS C18 column (150mm-2.1 mm, 5 μ m particle size) from United Chemical Technologies (UCT) was used for all separations. The mobile phase, which consisted of water with of 0.1% phosphoric acid (A) and acetonitrile (B), was used for gradient elution from 90A/10B to 100B in 10 min, then to 100B in 2.5 min. The flow rate was adjusted to 0.5 mL/min, the detection wavelength was 203 nm, and 2 μ L of sample was injected. All separations were performed at 45°C temperature. Peaks were assigned by spiking the samples with standard compounds and comparing retention times.

3 Results and discussions

Despite the fact that protodioscin is not very well absorbed in the ultraviolet spectral range, thanks to the ethane nitrile (acetonitrile) as a mobile phase, and the use of an analytical column with a small diameter, it was possible to create an analytical technique with a completely acceptable level of sensitivity for protodioscin (the minimum detection level is about 10 mg/L at a signal to noise ratio of 3/1). Protodioscin concentration dependence at the peak area is linear, with a high correlation coefficient (0.99992) as is shown in Figure 2.

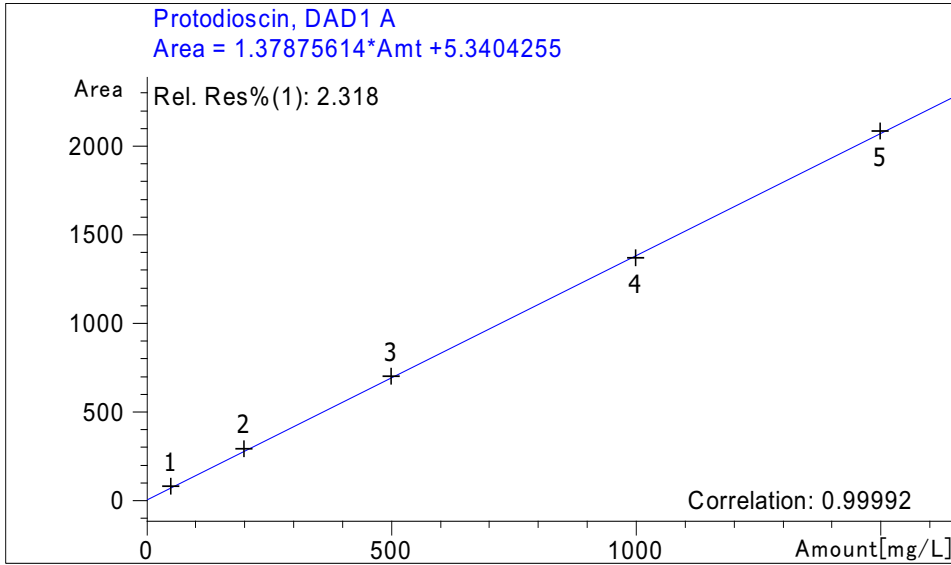


Fig. 2. Linearity test: Protodioscin concentration against the area of the chromatographic peak.

On Figure 3 and 4 There are represented raw materials chromatograms growing in the Republic of Crimea and Western Siberia.

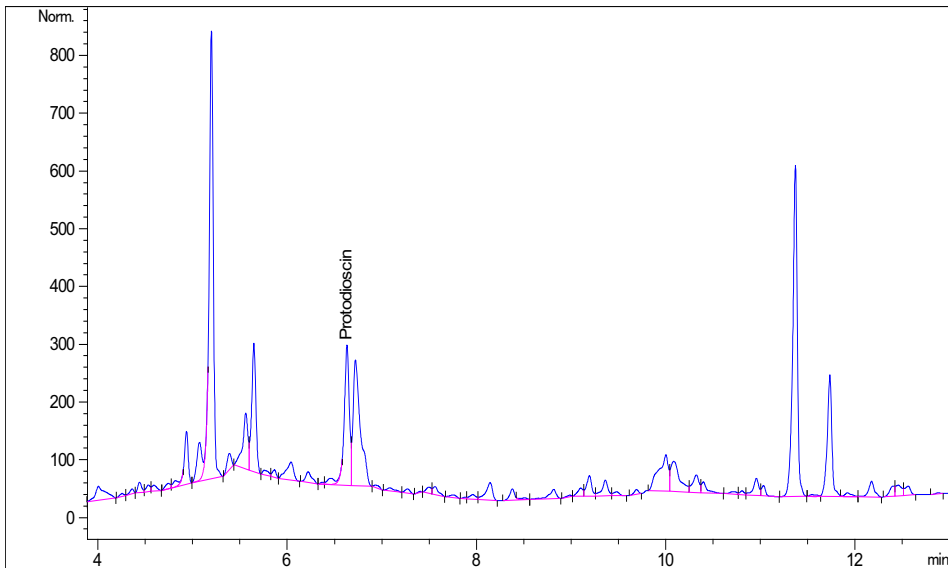


Fig. 3. Chromatogram of *Tribulus terrestris* sample (aerial parts from Crimea).

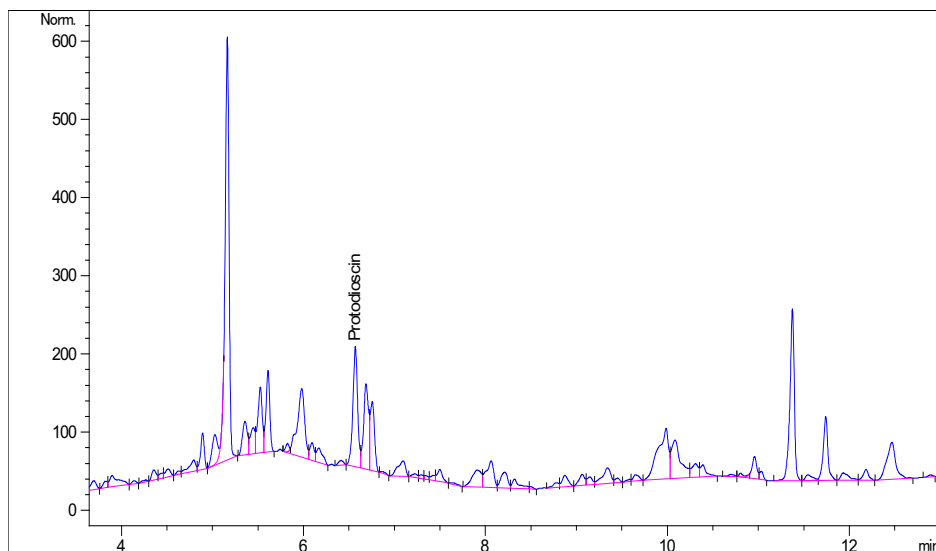


Fig. 4. Chromatogram of *Tribulus terrestris* sample (aerial parts from Western Siberia).

Method was used to analyze protodioscin analysis in two plant samples. Protodioscin peak from sample chromatograms was identified with retention time as compared with standard chromatogram. In raw material samples from Crimea was found: $0.546\% \pm 0.013$ protodioscin and in analyzed material from Western Siberia it was $0.338\% \pm 0.008$, for $n=3$ (Figure 5).

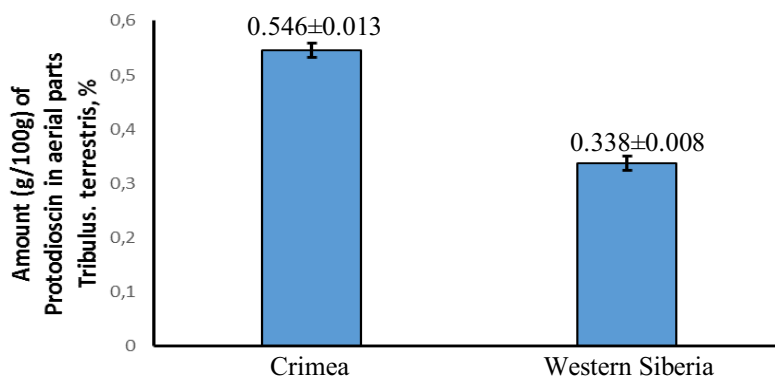


Fig. 5. Amount (g/100g) of Protodioscin in aerial parts *Tribulus Terrestris*.

The data presented in Figure 5 indicate that the raw materials grown on the region of Crimea are promising for the production of phytopreparations. The data obtained are well consistent with those available in the literature [9,16].

4 Conclusions

With high performance liquid chromatography protodioscin identification in the raw plants a simple survey has been developed. It allows in 15 minutes determine the protodioscin contents and other steroid compounds in the prepared plant extracts under conditions that they are purchased as standards.

Protodioscin content in the *Tribulus terrestris* aerial parts in studied samples growing in the Crimea is on average 60% higher than in similar raw materials from the West Siberian region. It is possible through favorable agro-climatic conditions on the peninsula. This fact provided an excellent opportunity for biologically active products containing steroid saponins from Crimean raw materials the production.

References

1. A. Affaf, Yu.N. Karpenko, D.K. Gulyaev et al., *Pharmacy & Pharmacology* **7(6)**, 279-290 (2019) doi: 10.19163/2307-9266-2019-7-6-346-355
2. X. Zhang, N. Wei, JU Huang, Y. Tan, D. Jing, *Natural Product Resource* **80(6)**, 67-71 (2011) doi:10.1080/14786419.2011.643886
3. G.K. Umarova, *Farmatsiya* **6**, 19-21 (2015) <https://www.elibrary.ru/item.asp?id=24131184>
4. M.N. Samy, M.M. Bishr, A.A. Ahmed et al., *Journal of pharmacognosy and phytochemistry zdb* **1**, 18-22 (2013) https://www.researchgate.net/publication/259296512_Pharmacognostical_Studies_on_Flower_of_Tribulus_terrestris_L
5. W. Zhu., Y. Du., H. Meng., Y. Dong., L. Li, *Chemistry Central Journal* **11**, 1–16 (2017) <https://doi.org/10.1186/s13065-017-0289-x>
6. A.M. Dirir, A.J. Cheruth, T. Ksiksi, *Journal of Natural Remedies* **17(2)**, 11043 (2017) doi:10.18311/jnr/2017/11043
7. T. Li, Z. Zhang, L. Zhang et al., *Journal of Separation Science* **32(23-24)**, 4167-4175 (2009) <http://dx.doi.org/10.1002/jssc.200900483>
8. O.I. Kozlova, O.I. Perederiaev, G.V. Ramenskaia, *Voprosy Pitaniia* **80(6)**, 67-71 (2011) https://www.researchgate.net/publication/223134429_Determination_by_high_performance_chromatography_steroid_saponins_in_a_biologically_active_food_supplements_containing_the_extract_of_Tribulus_terrestris
9. M. Shishovska, Z. Arsova-Sarafinovska, S. Memeti, *Journal of Chemical Engineering Research Updates* **2**, 12-21 (2015) https://www.researchgate.net/publication/275655812_A_Simple_Method_for_Determination_of_Protodioscin_in_Tribulus_Terrestris_L_and_Pharmaceuticals_by_High-Performance_Liquid_Chromatography_Using_Diode-Array_Detection
10. Y. Xu, Y. Liu, T. Xu et al., *Molecules* **15**, 613-618 (2010) doi:10.3390/molecules15020613
11. C. Saudan, N. Baume, C. Emery, E. Strahm, B. Saugy, *Forensic Science International* **178(1)**, 7-10 (2008) <http://dx.doi.org/10.1016/j.forsciint.2008.01.003>
12. M. Akram, H.M. Asif, N. Akhtar et al., *Journal of Medicinal Plant Research* **5(16)**, 3601-3605 (2011) https://www.researchgate.net/publication/260210811_Tribulus_terrestris_Linn_A_review_article
13. K. Milasius, M. Pečiukonienė, R. Dadeliene, J. Skernevičius, *Acta medica Lituanica* **17(1)**, 65-70 (2010) doi:10.2478/v10140-010-0008-6
14. A. Pokrywka, Z. Obmiński, J. Malczewska-Lenczowska, Z. Fijałek, *Journal of Human Kinetics* **41(1)**, 99-105 (2014) doi:10.2478/hukin-2014-0037
15. S.G. Angelova, Z. Gospodinova, M. Krasteva, G.G. Antov, *J. BioSci. Biotech* **2(1)**, 25-32 (2013) <https://www.researchgate.net/publication/290890305>
16. M. Ganzera, E. Bedir, I.A. Khan, *Journal of Pharmaceutical Sciences* **90(11)**, 1752-1758 (2001) doi: 10.1002/jps.1124