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CONTENT OF RUTIN AND STEROIDAL SAPONINS EXTRACTED FROM DIFFERENT GENOTYPES OF PUNCTURE VINE *TRIBULUS TERRESTRIS* L.

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Introduction

Puncture vine, *Tribulus terrestris* L., is an annual plant in the family *Zygophyllaceae* which is well adapted to grow in dry climate conditions (steppes, semi-desert, coastal sands, at road, on ruderal and gardens). It is occurs widely around the world from latitudes 35°S to 47°N [1, 2]. The genus *Tribulus* comprises about 20 species of creeping shrubs or herbs and the *Tribulus terrestris* is the most common among them. In the Europe, *Tribulus* grows in south-eastern part (Bulgaria, Romania, and Hungary). In Slovakia, *Tribulus* is found in southern regions [3]. It is characterized by the content of steroidal saponins.

Saponins are glucosides with foaming characteristics. They consist of a polycyclic aglycones attached to one or more sugar side chains. They have the ability to control the level of hormones in the human body. Many pharmaceutical preparations and food supplements with these saponins as the active compound have been commercially available [4].

Dioscin is known as an anti-cancer and anti-fungal phytochemical that has been shown to induce reactive oxygen-mediated apoptosis in several cancer cell lines and to alter cell membrane potential and membrane structure in yeast.

The main objective of this study is the investigation of content rutin and steroidal saponins (protodioscin, prototribescin and dioscin) in different puncture vine (*Tribulus terrestris* L.) genetic resources.

Material and methods

Plant material and experimental field

Four genotypes of puncture vine *Tribulus terrestris* from Slovakia, (Nové Zámky), Ukraine (Illichivs'k), Portugal (Lisbon) and Turkey (Belek) were used in this study.

Field experiments were conducted in the years 2012 and 2013, at the locality Presov, Slovakia (N 48° 59.382', E 21° 13.576', 253 m above sea level). Mean annual precipitation is 603 mm and mean annual temperature is 7.7 °C. The soil type at the experimental site is a fluvial soil (loam soil), pH = 7.2. The plots were size 5.0 m × 1.2 m, by flat 6.0 m^2 (for one experimental variant). The plants were growth in the greenhouse and planting in to the soil (early of May). The samples of plant (stems + leaves + flowers and fruits) were harvested at the first decade of September and naturally dried.

Extraction and HPLC analysis

The dried and powdered plant material (20 g) was extracted in 60 % v/v ethanol (reflux at 80ºC, 3 × 150 mL × 2 h). High-performance liquid chromatographic (HPLC) analysis was performed on a Varian Prostar 230 system. Measurements conditions were as follows: column Biospher PSI 200 C8 7 µm, 4.6×250 mm (Labio); flow rate 1 mL/min; injection volume 20 µL; mobile phase A: water/H_3PO_4 (99.5:0.5), B: 80 % acetonitrile; all solvents were gradient grade. The gradient (B in A) was 0 % isocratic for 5 min, linear from 0 to 100 % for 20 min, isocratic for 10 min, and finally from 100 to 0 % for 5 min. Samples were filtered through a 0.45 µm nylon syringe filter before analysis. Detection was performed using a UV detection system at 203 nm.

Statistical analysis

The statistical software Statgraphics 6.0 and multifactorial analysis of variance (ANOVA), method LSD 95 % (Least significant difference) was used for statistical analysis.

Results and discussion

The content of rutin and steroidal saponins are in the studied samples is presented in Table 1.

The highest content (3.95 %) of rutin was found in Slovak genotype and the lowest (1.12 %) in the Portugal genotype. Statistical evaluation of the content of this component has observed not significant difference between the evaluated genotypes (P = 0.500). Howeverm, statistically significant difference was found between samples from different experimental years (P = 0.048). Higher content of rutin was observed in 2013 (4.18 %) compared to year 2012 (0.29 %).

Table 1. Content of rutin and steroidal saponins in puncture vine (*Tribulus terrestris* L.) (% in DW) in extracted substances

Substance	Slovakia				Ukraine			
	Year		Average		Year		Average	
	2012	2013			2012	2013		
Rutin	0.40	7.50	3.95	±5.02	0.15	4.40	2.28	±3.01
Protodioscin	1.20	0.50	0.85	±0.40	3.20	0.40	1.80	±1.98
Prototribescin	0.60	0.50	0.55	±0.07	1.40	0.80	1.10	±0.42
Dioscin	0.60	0.40	0.50	±0.14	0.50	0.28	0.39	±0.16
Steroidal saponins-total	2.40	1.40	1.90	±0.71	5.00	1.50	3.25	±2.48

Substance	Portugal				Turkey			
	Year		Average		Year		Average	
	2012	2013			2012	2013		
Rutin	0.23	2.00	1.12	±1.25	0.38	2.80	1.59	±1.71
Protodioscin	2.70	1.00	1.85	±1.20	2.40	1.00	1.70	±0.99
Prototribescin	1.00	0.50	0.75	±0.35	1.30	0.50	0.90	±0.57
Dioscin	0.60	0.30	0.45	±0.21	1.10	0.28	0.69	±0.58
Steroidal saponins-total	4.40	1.80	3.10	±1.84	4.80	1.70	3.25	±2.19

Average contents of protodioscin in years 2012 and 2013 ranged from 0.85 % to 1.85 %, prototribescin from 0.55 to 1.15 % and dioscin from 0.39 - 0.69 %. Statistical evaluation has observed not significant difference in content of this steroidal saponins between the experimental years and genotypes.

Authors of [5] have analysed the five samples of puncture vine. Two cultivated (genotypes from Hungary and Turkey) and three wild genotypes from Bulgaria (Plovdiv and Vedrare). It was found, that content of rutin from puncture vine cultivated in Turkey (0.15 %) and Hungary (0.10 %) is ten times higher than in samples from wild population from Bulgaria (from 0.01 % to 0.03 %). The samples from Bulgaria contain high amount of protodioscin and prototribescin. These furanostal saponins were dominant in the sample from Plovdiv wild population (protodioscin 2.48 %; prototribescin 1.50 %). Content of dioscin in wild populations varied from 0.60 % (Vedrare) to 0.85 % (Plovdiv). In cultivated samples were content of this compound from 0.47 % (Turkey) to 0.75 % (Hungary). Authors of [6] have reported the content of rutin - 0.03 %, protodioscin - 1.70 %, prototribescin - 1.38 % and dioscin - 0.62 % in the sample from cultivated in Bulgaria (Sofia, genotype from Plovdiv). The content of protodioscin in leaves 1.34 %, stems 0.28 % and fruits 0.25 % (samples from Bulgaria) was reported by authors of [7].

Authors of [8] have reported, that increasing levels of water stress progressively reduced vegetative growth and biomass production and increased carbohydrate, soluble sugars and free proline content. The chemical concentrations of steroidal saponins increased under water stress conditions. Also our experiments shoved, that soil and climatic condition have influence to accumulation of biologically active components.

Analysis of phytochemicals variability between accessions in the case of medicinal plants will help to identify them in terms of their differences in phytochemical constitution. Moreover, phytochemical differences are good indicators of their genotypic distances [9].

Conclusion

Significant differences in the accumulation of rutin and steroidal saponins were not observed for genotypes from different countries. The obtained results show the significant impact of experimental year on the accumulation of biologically active substances. The accumulation of content of various substances in the Slovakian and Ukrainian genotypes is affected by infestation of downy mildew Hyaloperonospora (*Peronospora Tribulina*). The disease has destroyed the above ground part of plants which then has regenerated. Therefore, the highest content of rutin in Slovak genotype (3.95 %) was found in 2013.–High content of rutin was recorded also for Ukrainian genotype (2.28 %). In contrast, the content of rutin was lower in Portugal (1.12 %) and Turkish (1.59 %) genotypes.

The highest content of total steroidal saponins was found in Ukrainian and Turkish genotypes (3.25 %). High content of total steroidal saponins was recorded also in Portugal genotype (3.10 %). Slovak genotype has accumulated least amount (1.90 %).

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In the present study we were evaluated four plant populations of Puncture vine *Tribulus terrestris* L. from Slovakia (Nové Zámky), Ukraine (Illichivs'k), Portugal (Lisbon) and Turkey (Belek). These plant resources were tested during the years 2012 - 2013 in small plot trials on experimental fields in Presov (the North - Eastern part of Slovakia). The biologically active compounds rutin and steroidal saponins (protodioscin, prototribescin and dioscin) were extracted by 60 % ethanol. The extract contains (HPLC) 1.12-3.95 % of rutin, 0.85-1.85 % of protodioscin, 0.55-1.15 % of prototribescin and 0.39-0.69 % of dioscin. The highest content (3.95 %) of rutin was found in Slovak and the lowest (1.12 %) in the Portugal genotype.