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Research Article

Analysis of diosgenin and phenol compounds in *Tribulus terrestris* L.

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Abstract

Caltrop (*Tribulus terrestris* L.) is a weed plant widely growing in warm climate. The crude caltrop has multidirectional pharmacological action favorable for the development of drug preparations. As in European countries caltrop is a non-officinal plant, it seems feasible to develop raw material standardization parameters for creation of novel drugs. In the course of thin layer chromatography research the following compounds were identified in caltrop herb purveyed during the fruiting season: rutin, chlorogenic acid, caffeic acid, diosgenin. Diosgenin content in this raw material was determined by densitometry to be min. 0,11%.

Keywords

caltrop, herb, TLC, densitometry

Introduction

Tribulus terrestris L. (caltrop) is an annual plant of *Zygophyllaceae* family.

Caltrop grows in warm and tropical areas of Europe, Asia, Africa and Australia (Sasikala et al. 2014; Chhatre et al. 2014).

Plant chemical composition is represented mostly by steroid saponins, such as protodioscin, dioscin, gracilin, protogracilin, diosgenin, gitogenin, ruscogenin, gecogenin, chlorogenin, tygogenin, sarsasapogenin (Xu et al. 2010; Abirami and Rajendran 2011; Sidjimova et al. 2011; Kumar and Bhardwaj 2012; Jameel et al. 2012; Hammoda et al. 2013; Chhatre et al. 2014). It also contains some flavonoids: rutin, astragalin, tribuloside, quercetin, kaempferol, isorhamnetin, luteolin (Raja and Venkataraman 2011; Kumar 2012; Noori et al. 2012; Patil et al. 2012; Renuka et al. 2012; Yanala and Sathyanarayana 2018).

As the crude caltrop shows versatile pharmacological activities, including anti-inflammatory, anti-sclerotic, diuretic, restorative, hepatoprotective, anti-microbial, anti-tumor, anti-spasmodic activities, it is used to cure various diseases in folk medicine of numerous countries (Jameel et al. 2012; Sasikala et al. 2014; Chhatre et al. 2014). Thus, this plant has prospects for the development of drugs on its basis.

Nevertheless, caltrop herb is not included in Pharmacopoeia in Ukraine and other countries, therefore, it would be feasible and necessary to develop its standardization parameters.

Objective of the present work was detection of phenol compounds and diosgenin in caltrop herb by TLC as well as quantitative determination of diosgenin content in this raw material.

Materials and methods

Caltrop herb was purveyed for research during fruiting season of 2016–2018 in southern areas of Ukraine.

TLC detection of phenol compounds. To 0,5 g of raw material 5 ml of methanol was added, it was put to reflux water bath for 5–10 minutes, cooled and filtered.

Reference solution: standard sample of caffeic acid (2,7 mg), chlorogenic acid (1,22 mg), hyperoside (3,0 mg), rutin (1,5 mg) dissolved in 5 ml methanol.

10 μL of investigative solution and reference solution were smeared on a chromatogram.

Plate: TLC plate with silica gel layer F_{254} Merck.

Mobile phase: formic acid – purified water – methyl ethylketone – ethyl acetate (10:10:30:50).

Detection: UF light, wavelength 365 nm after treatment with diphenylboric acid aminoethyl ester solution in methanol and with macrogol solution, plate dried at 105 °C

Diosgenin analysis

TLC detection of diosgenin. 5 g of crushed raw material was placed into a round-bottom flask, 30 ml sulfuric acid solution was added, the mixture was boiled on a reflux water bath for 3 hours, cooled, filtered, then the filter with a raw material was washed up to a neutral reaction.

The filter with residue was put into a conical flask, 30 ml methanol was added, and the mixture was subjected to ultrasonic treatment for 30 min. Then it was placed into 50 ml volumetric flask. 15 ml methanol more was added to the residue under ultrasonic treatment.

The obtained mixture was filtered into the same volumetric flask and replenished with methanol up to the mark. 5 ml of the obtained solution was evaporated to dry residue and dissolved in 1 ml methanol.

Reference solution: 5 mg standard diosgenin sample was dissolved in 10 ml methanol.

10 μL experimental solution and reference solution were smeared on a chromatogram.

Plate: TLC plate with silica gel layer F_{254} Merck.

Mobile phase: methanol – methylene chloride (5:95).

Detection: day light, after treatment with anisaldehyde, 105 °C.

Diosgenin content determination. 5 g of crushed raw material was placed into a flat bottom flask, 50 ml chloroform added, covered and treated for 30 min with ultrasound, then filtered. The filter residue was dried in a cabinet drier at 50 °C.

1 g of dried material was placed to a round bottom flask and 20 ml 15% sulfuric acid solution was added, then it was boiled on a water bath up to pH paper neutral reaction.

The filter with residue was put into a conical flask, 40 ml methanol was added, the mixture was subjected to ultrasonic treatment for 30 min. Then it was filtered into 50 ml volumetric flask. 10 ml methanol more was added to the residue in a conical flask, mixed, then filtered to the same 50 ml volumetric flask and replenished with methanol up to the mark.

25 ml of the obtained solution was evaporated on a water bath up to dry residue and the residue was dissolved in 1 ml methanol.

Reference solution: 1 mg standard diosgenin sample was dissolved in 2 ml methanol.

10 μL experimental solution and 5 μL, 10 μL, 15 μL reference solution were smeared on a chromatogram.

Plate: TLC plate with silica gel layer F_{254} Merck.

Mobile phase: the bottom layer of a mixture of chloroform-methanol-water (26: 14: 4) defend at a temperature below 10 °C for 5 hours.

Detection: day light, after treatment with anisaldehyde, 105 °C.

The quantitative content of diosgenin was determined by densitometry using Sorbfil program.

Results and discussion

Fig.1 shows the sequence of fluorescent areas in reference solution chromatogram and experimental solution chromatogram under study of phenol compounds.

The chromatographic study of phenol compounds in caltrop herb relative to their Rf value and fluorescence intensity revealed the presence of rutin, chlorogenic acid and caffeic acid.

Fig. 2 shows the sequence of areas in chromatogram of reference solution and experimental solution in detection of diosgenin.

Thus, diosgenin was identified in caltrop herb.

Fig. 3 shows the sequence of areas in chromatogram of reference solution and experimental solution in determination of diosgenin content in caltrop herb.

In accordance with the ICH and the State Pharmacopoeia of Ukraine, this methodology was validated.

Validation of the proposed methodology was carried out according to the following parameters: specificity, system suitability testing, detection limit, linearity and repeatability.

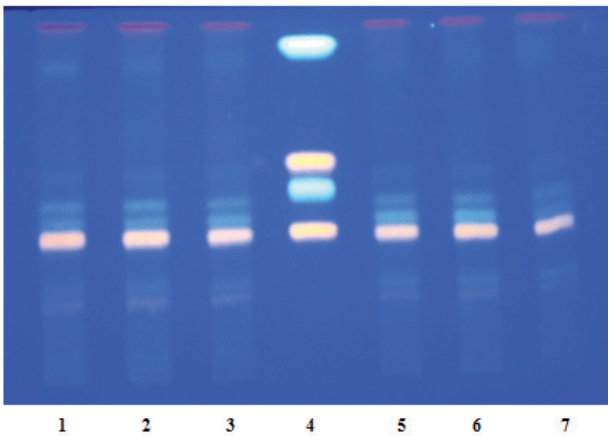

Figure 1. Chromatogram of phenol compounds determination in caltrop herb. **1, 2, 3, 5, 6, 7** – solutions of caltrop herb samples, **4** – solution of standard samples of substances (caffeic acid, chlorogenic acid, rutin, hyperoside).

Figure 2. Diosgenin identification chromatogram in caltrop herb. **1** – diosgenin standard sample, **2**- solution of caltrop herb sample.

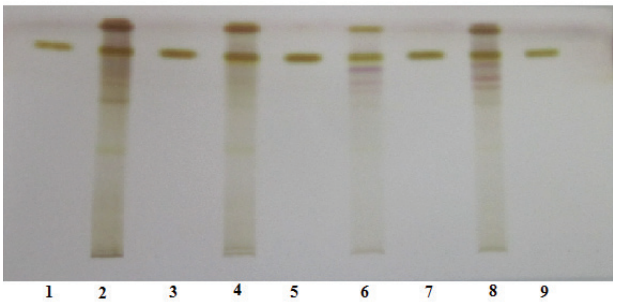

Figure 3. Diosgenin quantitative determination chromatogram in caltrop herb. **1** – diosgenin standard sample (5 µL / 2,5 µg); **3** – diosgenin standard sample (10 µL / 5 µg); **5** – diosgenin standard sample (15 µL / 7,5 µg); **7** – diosgenin standard sample (10 µL); **9** – diosgenin standard sample (5 µL); **2, 4, 6, 8** – solutions of caltrop herb samples.

The specificity was determined by the value of the Rf spot of the control track, which should correspond to the Rf spot of the standard sample (Rf = 0,82±0,01).

The efficiency of the plate was determined by the asymmetry coefficients (As) peaks in the chromatograms. As for solutions of a standard sample of diosgenin was 0.85–1.0; for solutions of caltrop herb samples – 1.12–1.15.

If the asymmetry coefficient does not exceed 2–2.5, then the distortion of the peak shape does not greatly affect the quality of separation in TLC (Gavrilin and Senchenko 2008).

The analog curves of the standard solution of diosgenin and solution of caltrop herb are shown in Figs 4, 5.

Under these conditions, neither the solvent nor the mobile phase interferes with the determination of the active substance.

The sensitivity of the methodology was determined by the magnitude of the detectable minimum of the substance in the spot, which was visually manifested after detection ($2.5^{*}\ 10^{-6}$ g).

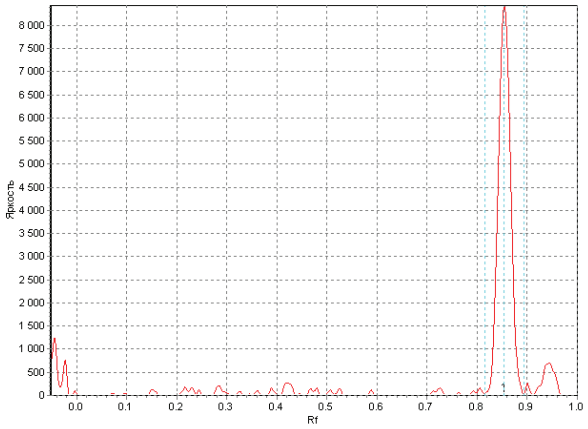

Figure 4. Analog curve of the standard solution of diosgenin.

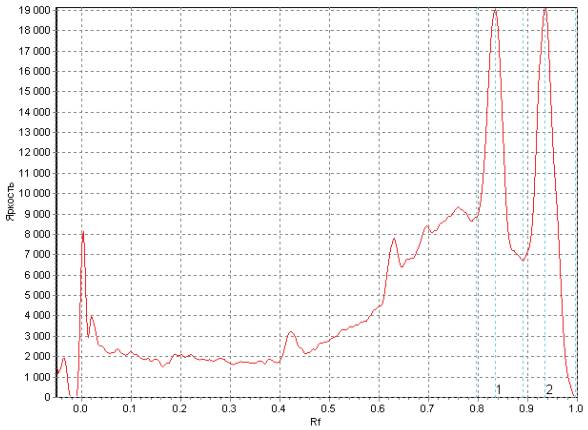

Figure 5. Analog curve of the solution of caltrop herb.

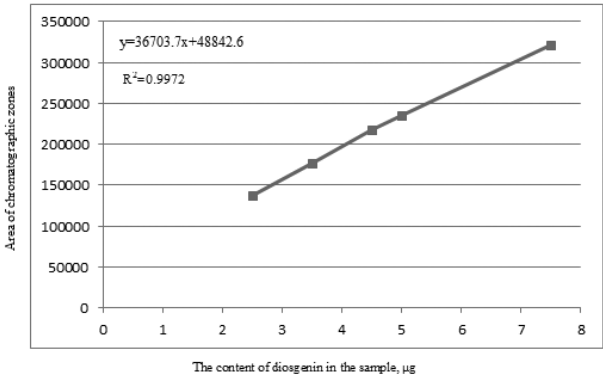

Figure 6. The dependence of the area of the chromatographic zones on the content of diosgenin in the sample.

A linear relationship was established between the content of diosgenin and the area of the chromatographic zone in the range of studied concentrations (Fig. 6).

The intermediate precision of the methodology was determined. The results obtained by statistical processing are reliable at a confidence level of 95%, which indicates the accuracy of the method under repeatability conditions.

The metrological assessment of the proposed methodology is presented in Table 1.

The study determined that diosgenin content in caltrop herb must be min. 0,11%.

Table 1. Metrological characteristics of the analysis methodology.

f	X_m	S^2	S	P, %	t(P,f)	Δx	ε, %
4	0.144	0.000030000	0.0025	0.95	2.78	0.0068096	4.72

Conclusions

The TLC analysis of phenol compounds and diosgenin in caltrop herb permitted to identify rutin, chlorogenic acid and caffeic acid, as well as diosgenin. In addition, the densitometry determined diosgenin quantitative content to be min.0,11%. For this methodology, validation indicators were identified: specificity, system suitability testing, detection limit, linearity and repeatability.

The obtained experimental data will be used in the development of caltrop herb standardization parameters.

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