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Research Article

Identification and quantitative analysis of furostanol glycosides in caltrop

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Abstract

This study describes the identification and quantitative determination of furostanol glycosides in caltrop herb harvested in the fructification period. Furostanol glycosides were identified by thin-layer chromatography (TLC) and quantified by UV-vis spectrophotometry. The furostanol glycosides were visualized on the TLC plate as pink spots after treatment with dimethylamine benzaldehyde solution. UV-vis spectrophotometry quantified these substances to the amount of at least 0.4 %. The data on the identification and quantification of furostanol glycosides obtained in the course of this research was implemented in the development of the Ukrainian State Pharmacopoeia for caltrop herb.

Keywords

caltrop, herb, TLC, UV-vis spectrophotometry

Introduction

Tribulus terrestris L. (caltrop) is an annual plant belonging to the Zygophyllaceae family. Its procumbent stem may reach up to 1 m. The leaves are compound, paripinnate, small, elongated, and feathery. The flowers are small and yellow. The fruits are dry nuts with sharp spikes. The plant grows in warm and tropical areas of Europe, Asia, Africa and Australia. In Ukraine, caltrop is encountered in the southern regions (Raina et al. 2007; Mohd et al. 2012; Chhatre et al. 2014; Shishovska et al. 2015).

The caltrop herb contains the steroid saponins: protodioscine, dioscine, prototribestin, pseudoprotodioscin, tribestin, tribulosin, gracillin, protogracillin, diosgenin, gitogenin, ruscogenin, gecogenin, tigogenin, chlorogenin and sarsasapogenin (Dinchev et al. 2008; Mohd et al. 2012; Hammoda et al. 2013; Kang et al. 2014; Shishovska et al. 2015). Besides them, its components are also flavonoids, tannins and nitrogen-containing substances (Sidjimova et al. 2011; Kumar and Bhardwaj 2012).

The crude caltrop extracts exhibits various pharmacological activities, including anti-inflammatory, anti-sclerotic, analgesic, diuretic, tonic, antispasmodic, prostate protective, anti-microbial, expectorant, anti-tumor, anti-helminthic effects (Benzie et al. 2011; Mohd et al. 2012; Hammoda et al. 2013; Chhatre et al. 2014).

Monographs on caltrop fruits exist in Japanese, Indian Ayurvedic and Chinese Pharmacopoeia (Pharmacopoeia of the People's Republic of China 2005; The Ayurvedic Pharmacopoeia of India 2008; JP XVII 2016). Roots are

also described in Indian Ayurvedic Pharmacopoeia (The Ayurvedic Pharmacopoeia of India 2008). However, the herb of this plant is not a pharmacopoeia herb. The European Pharmacopoeia also does not contain any reference to caltrop materials. As the State Pharmacopoeia of Ukraine is totally harmonized with the European Pharmacopoeia, any criteria for standardization of this object in Ukraine still does not exist.

Therefore, for the development of national monograph on caltrop herb in the State Pharmacopoeia of Ukraine it is necessary to perform a standardization of this material on basic active substances, namely, furostanol glycosides.

The purpose of our work was the development of methods for furostanol glycosides identification, as well as their quantification in caltrop herb in order to create standardization criteria.

Materials and methods

Plant material

The caltrop plant was harvested in 2016–2018 in the fructification period (June–August) in southern areas of Ukraine.

For furostanol glycosides identification and quantification in caltrop plant we developed analytical methodologies considering the peculiarities of the material under study.

Identification of furostanol glycosides in caltrop herb

Furostanol glycosides were identified in caltrop herb by thin-layer chromatography (TLC).

Sample preparation: To 1.0 g of raw material 10 ml of 80% ethanol were added, the mixture was heated at 60 °C on reflux water bath for 15 minutes, cooled, filtered and evaporated to 1 ml volume. This loading (with the help of 0.5 ml water) was moved to a separatory funnel, extracted twice with 1 ml aliquots of chloroform, and then the chloroform extracts were discarded.

Reference solution: 0.2 g of caltrop dry extract was dissolved in 5 ml methanol.

Plate: Silica gel F_{254} Merck, size 20*10 cm on glass substrate.

After sample application the plate was dried on air for about 5–10 minutes and put in a chamber.

When the solvent reached the plate front, the TLC chromatogram was taken out, dried on air and spots were observed after sprinkling with *p*-dimethylamine benzaldehyde solution and then heating at 105 °C.

Mobile phase: Mixture of solvents: chloroform : methanol : water : anhydrous formic acid (61:32:7:0.5).

Sample volume: Experimental solution 10 µl, reference solution 15 µl.

Developer reagent: *p*-dimethylamine benzaldehyde solution (0,5 g *p*-dimethylamine benzaldehyde was dissolved in 17 ml concentrated hydrochloric acid and 50 ml methanol), heating was done at 105 °C.

Quantification of furostanol glycosides in caltrop herb

Furostanol glycosides were quantified by UV-vis spectrophotometry.

Sample preparation: To a conical flask, an aliquot of 0.5 g of ground caltrop herb was put and extracted with 50 ml methanol 30 minutes on ultrasonic bath at room temperature. The obtained extract was filtered by filter paper to a round-bottom flask. The extraction was repeted: 20 ml methanol was added to the raw material residue, ultrasonicated for 15 minutes and filtered by filter paper to a round-bottom flask. The flask content (the combined methanol extract from the first and the methanol extract from the second extraction) was evaporated to dryness and then 20 ml 3 M hydrochloric acid solution was added and ultrasonicated until complete dissolution for 15 minutes at room temperature. The obtained solution was heated at 60 °C on reflux water bath for 30 minutes and cooled. Then 30 ml of chloroform were added and heated at 60 °C on reflux water bath for another 15 minutes. The mixture was cooled and the chloroform layer was separated on a separatory funnel. The water layer was returned back to round-bottom flask, 30 ml chloroform were added to it and the mixture was heated at 60 °C on reflux water bath for 15 minutes. The solution was cooled, transferred to a separatory funnel and the chloroform layer was separated. The chloroform extracts were combined, filtered through filter paper into a round-bottom flask, and the filter was washed with chloroform. The combined chloroform extracts were evaporated to dryness. To the residue, were added 50 ml of methanol in portions, the mixture was transferred to a graduated flask and methanol was added up to the mark of 50 ml. 0.5 ml of the obtained solution was taken to a screw-top vial, boiled to dryness The dry extract was dissolved by ultrasonication with 2 ml ethyl acetate. Further, 1 ml of solution A and 1 ml of solution B were mixed stirred, closed with cover and maintained at 60 °C for 20 minutes. The obtained solution was quickly cooled to room temperature under running water and after 15 minutes its absorbance was measured at 430 nm wavelength on Mecasys Optizen POP spectrophotometer.

Preparation of solution A: 0.5 ml anisic aldehyde was diluted in 100 ml graduated flask with ethyl acetate and added up to the mark.

Preparation of solution B: 25 ml sulfuric acid was mixed with 25 ml ethyl acetate.

Preparation of reference solution: 0.015 g standard diosgenin sample was dissolved in 50 ml graduated flask and with 50 ml methanol, the latter being added up to the mark. 5.0 ml of the obtained reference solution was pipette into 25 ml graduated flask, and methanol was added up to the mark. 0.5 ml from the obtained solution was poured to a screw-top vial, boiled to dryness. Then 2 ml ethyl acetate were added to the dry extract and it was dissolved with the help of ultrasound. Further 1 ml of solution A and 1 ml of solution B were added, stirred, closed with a stopper and maintained for 20 minutes at 60 °C. The solution was

quickly cooled to room temperature under running water and after 15 minutes the absorbance was measured at wavelength 430 nm.

Preparation of compensation solution: 2 ml ethyl acetate was put to screw-top vial, 1 ml of solution A and 1 ml of solution B were added, maintained for 20 minutes at 60 °C and quickly cooled to room temperature under running water.

The content of furostanol glycosides (X, %) as diosgenin equivalents was calculated by the formula:

$$X = \frac{A_1 \cdot 50 \cdot 0,015 \cdot 5 \cdot 0,5 \cdot 100 \cdot 100}{A_0 \cdot m_2 \cdot 0,5 \cdot 50 \cdot 25 \cdot (100 - W)},$$

A_1 – absorbance of tested solution;
A_0 – absorbance of reference solution;
m_2 – mass of tested raw material aliquot, g;
W – moisture content in raw material, %.

Results and discussion

The TLC analysis of caltrop herb revealed the furostanol glycosides area as pink spots (Fig. 1).

In accordance with the ICH and the State Pharmacopoeia of Ukraine, the proposed methodologies on iden-

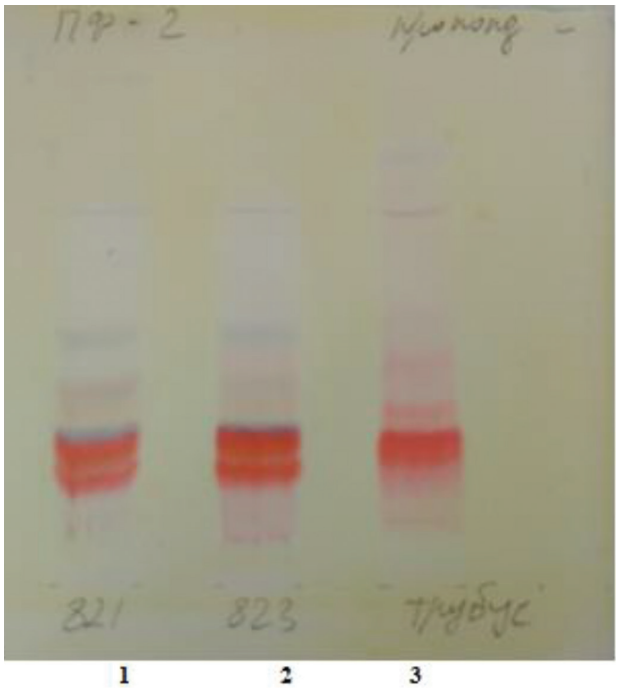
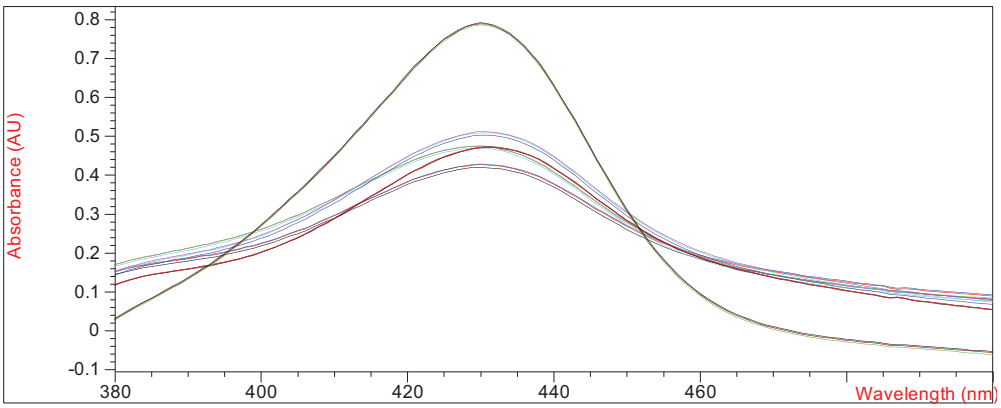
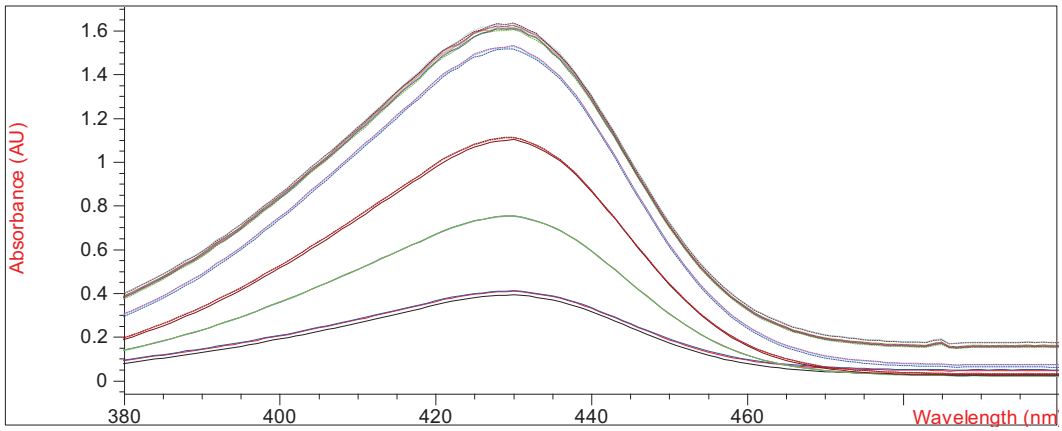


Figure 1. Chromatogram of caltrop herb furostanol glycosides. Note. 1, 2 – caltrop herb solution samples, 3 – caltrop dry extract solution (pharmacopoeia standard).



"#"	"Name"	"Abs<430nm>"
1	"815/1"	0.419457
2	""	0.426332
3	""	0.42801
4	"815/2"	0.43953
5	""	0.433564
6	""	0.434401
7	"818/1"	0.459174
8	""	0.460552
9	""	0.460016
10	"818/2"	0.482059
11	""	0.487745
12	""	0.481324
13	"diocgenin"	0.785661
14	""	0.788054
15	""	0.790818

Figure 2. UV-vis spectra of caltrop herb samples and standard diosgenin sample. Note. 1, 2, 3, 4 – caltrop herb series; diosgenin – standard sample.



"#"	"Name"	""Abs<430nm>"
1	"diocgenin 0.25 ml"	0.393867
2	"25 µg"	0.409621
3	""	0.412474
4	"0.5 ml"	0.753004
5	"50 µg"	0.754479
6	""	0.754166
7	"0.75 ml"	1.104052
8	"75 µg"	1.110566
9	""	1.108067
10	"1.0ml/ 14:37"	1.513609
11	"100 µg"	1.523314
12	""	1.5285
13	"14:40"	1.602482
14	""	1.630937
15	""	1.604244
16	"14:50"	1.611115
17	""	1.60896
18	""	1.629241
19	"14:55"	1.620548
20	""	1.612212
21	""	1.620594

Figure 3. UV-vis spectra of standard diosgenin solution samples.

tification and quantification of furostanol glycosides in caltrop herb are validated.

For quantification methodology, validation indicators were identified: accuracy, precision and linearity.

UV-vis spectra of caltrop herb series are specified in Fig. 2.

For determining the linearity of the methodology, standard diosgenin solution samples absorbance were measured (Fig. 3).

Diosgenin absorbance is stable when measured 15 minutes after preparation of the solution.

A linear relationship was established between the content of diosgenin and absorbance in the range of the studied concentrations (Fig. 4).

The quantification study of the sum of furostanol glycosides as diosgenin equivalents in caltrop herb showed their content at a level minimum of 0.4 %.

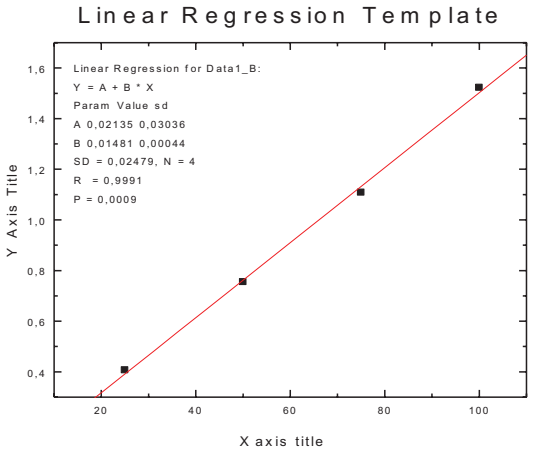


Figure 4. The graph of dependence of diosgenin solutions absorbance on its concentration.

Conclusions

Some furostanol glycosides from harvested in Ukraine caltob herb were identified using the discussed TLC method as methanol extracts. The substances were identified as pink spots. The quantity of diosgenin equivalents were determined by UV-vis spectrophototmetry.

The proposed research methodology and the obtained results on identification and quantification of furostanol glycosides in caltrop herb were implemented in the development of national monograph on the caltrop herb in the State Pharmacopoeia of Ukraine.

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