

HPTLC QUALITATIVE AND QUANTITATIVE DETECTION OF STEROLS IN SPECIES OF THE *PORTULACA* GENUS FROM ROMANIA

ADRIANA IULIANA ANGHEL¹, MIHAELA ILIE², OCTAVIAN TUDOREL OLARU^{1*}, MIHAELA DINU¹, ROBERT VIOREL ANCUCEANU¹, VIORICA ISTUDOR³

“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, 6 Traian Vuia Street, 020956, Bucharest, Romania

¹Pharmaceutical Botany Department

²Toxicology Department

³Pharmacognosy, Phytochemistry and Phytotherapy Department

*corresponding author: octav_olaru2002@yahoo.com

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Abstract

Sterols are secondary metabolites of plants, known for their inhibitory effects on stomach, lung, breast and ovarian cancer. The intake of phytosterols can enhance the activity of antioxidant enzymes, thus reducing the oxidative stress. The species of the *Portulaca* genus from Romania have not been analysed until now for their sterols content. We hereby report on the qualitative and quantitative analysis of the sterols from *Portulaca oleracea* L. and *Portulaca grandiflora* Hook. in view of their potential therapeutical use.

Alcoholic extracts were obtained from herbal parts of *Portulaca grandiflora* and *Portulaca oleracea*, and the HPTLC analysis was applied to these extracts, using silica gel 60 F₂₅₄ plates and chloroform – acetone (80:20) as mobile phase; the densitometric analysis was performed at 206 nm. The sterol amount contained in each sample was calculated and expressed as β -sitosterol, which was used as external standard. Based on the comparison of R_f values and of the spectra of the spots with those of the standard, β -sitosterol was identified in both samples. The HPTLC quantitative analysis of the methanolic extracts showed as much as 79.9 mg β -sitosterol per 100 g of herbal product from *Portulaca grandiflora* and 73.1 mg per 100 g of herbal product from *Portulaca oleracea* (on a dry basis).

Rezumat

Sterolii sunt metaboliți secundari ai plantelor, cunoscuți pentru efectele lor inhibitorii asupra cancerului ovarian, de sân, pulmonar și gastric. Aportul de fitosteroli poate stimula activitatea enzimelor antioxidante, reducând astfel stresul oxidativ. Speciile genului *Portulaca* din România nu au fost analizate până acum pentru conținutul lor în steroli. Articolul de față prezintă rezultatele analizei calitative și cantitative a sterolilor din *Portulaca oleracea* L. și *Portulaca grandiflora* Hook., în vederea potențialelor lor valorificări terapeutice. S-au obținut extracte alcoolice din părțile aeriene ale *Portulaca grandiflora* și *Portulaca oleracea*, și s-a aplicat analiza HPTLC acestor extracte, utilizând plăci cu silicagel 60 F₂₅₄ și cloroform-acetonă (80:20) ca fază mobilă; analiza densitometrică a fost efectuată la 206 nm. Conținutul în steroli din fiecare probă a fost calculat utilizând β sitosterolul ca standard. Pe baza comparației valorilor R_f și a spectrelor spoturilor cu cele ale standardului, β sitosterolul a fost identificat în ambele probe. Analiza cantitativă HPTLC a extractelor metanolice a evidențiat un conținut de 79,9 mg β -sitosterol la 100 g produs vegetal provenit de la *Portulaca grandiflora* și 73,1 mg la 100 g de produs vegetal provenit de la *Portulaca oleracea* (raportat la produsul uscat).

Keywords: *Portulaca*, β -sitosterol, HPTLC

Introduction

Plant sterols, also known as phytosterols, are secondary metabolites characterized by a cyclopentanoperhydro-phenanthrene structure, resembling to the animal-specific cholesterol structure. Similarly to the latter, sterols may exist in a free form or in an esterified one. Some of the most common free plant sterols are sitosterol, stigmasterol, avenasterol [8]. It is generally agreed that a daily dose of 1-3 g of phytosterols reduces by 10-15% the level of total cholesterol and of LDL-cholesterol [9].

Experimental data (*in vitro* tests, animal xerograft models and epidemiological studies) support an inhibitory effect of phytosterols in various forms of cancer (stomach, lungs, breast and ovarian and others) [2, 3]. Several mechanisms of actions have been proposed, one of them being the triggering of the apoptosis in cancer cells. The phytosterols intake can also enhance the activity of antioxidant enzymes, thus reducing the oxidative stress [16]. Although the phenolic compounds are well-known for their antioxidant action [12], in the last years a series of studies evaluated this action for

phytosterols as well, explaining it on the basis of their molecular structure [13].

The presence of free and glycosylated sitosterols has been reported in species of the *Portulaca* genus [5, 13]. No method of quantitative or qualitative detection of sterols through thin layer chromatography (TLC) or high performance thin layer chromatography (HPTLC) could be found in the studied literature, although this is widely used for the detection of various compounds in mixtures [6] and plant extracts, as it is considered faster, simpler and cheaper than other methods [14, 15]. We therefore used thin-layer chromatography (TLC) and HPTLC for the analysis of sterols in the *Portulaca* species: TLC for the qualitative detection and HPTLC coupled with densitometry for the quantitative assay.

Materials and Methods

The herbal material used was the dried and comminuted herb from *P. oleracea* L. (Po) and *P. grandiflora* Hook. (Pg) species, harvested from Bucharest (several private gardens). Beta-sitosterol was acquired from Sigma-Aldrich and the other reagents and solvents from various European suppliers (Scharlau Chemie SA, Chemical Company, Chemopar SA). Amounts of about 1 gram of each herbal product were refluxed for 30 minutes with 50 mL methanol, each. Methanolic extracts thus obtained were subsequently analysed through TLC and after a good separation was thus obtained, the method was applied on HPTLC plates, using a semi-automated spotting system, as described below.

A stock solution of 1mg/mL β -sitosterol was prepared by dissolving the reference substance in chloroform. This stock solution was diluted three times to obtain the reference solution applied for HPTLC.

From the reference solution, volumes between 0.5 and 2 μ L were applied on the chromatographic plate. The samples, represented by the methanolic extracts from the two herbal products, were spotted alternatively with the standard in two different amounts: 10 μ L and 20 μ L. The application of the spots was carried out in bands, under a nitrogen flow, using a LINOMAT 5 (CAMAG – Switzerland) device (a semi-automated spotting system); bands of 10 mm length were applied with a 11.3 mm distance between spots, on standard pre-coated silica gel 60 F₂₅₄ glass plates, 20x10cm (Merck). The mobile phase used was chloroform – acetone (80:20) (v:v) [10].

After developing in a vertical glass tank, previously saturated with the vapours of the mobile phase, the plates were left still to dry and densitometrically analysed at 206 nm [1, 7] via a TLC SCANNER 3 (CAMAG – Switzerland) UV-VIS densitometer.

For revelation we used the derivatizing agents acetic anhydride and sulfuric acid: ethanol mixture

(1:1) (v:v), then the chromatographic plate was kept for 5 minutes at 105°C.

The data analysis was performed with a WinCATS Planar Chromatography Manager software, version 1.4.4.6337 (CAMAG – Switzerland).

Results and Discussion

The separation of β -sitosterol through HPTLC, using the silica gel as stationary phase and the chloroform – acetone mixture (80:20) as mobile phase lead to good results for the samples, the chromatogram showing no "tails" and the front being linear. Representative chromatograms of the samples and standard after revelation are presented in Figures 1-2. The mean and the standard deviation based on three chromatograms were calculated for the computed R_f values and the results are being presented in Table I.

Table I

The average values of the R_f computed for the samples and for the standard

No.	Standard/sample	Mean R_f	SD
1	Standard- β sitosterol	0.9077	0.0117
2	<i>P. oleracea</i> (Po)	0.9025	0.0050
3	<i>P. grandiflora</i> (Pg)	0.9075	0.0096

Mean = arithmetic mean (average); SD = standard deviation;
Rf = retention factor

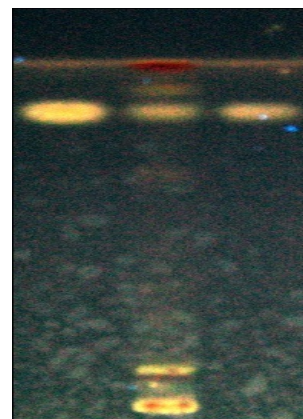


Figure 1.

Chromatogram with the spots: standard_Po_standard

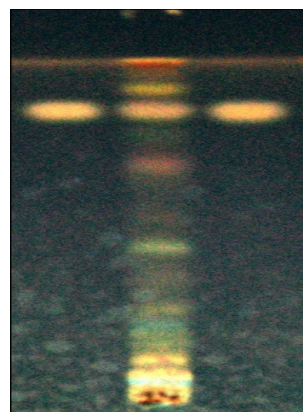


Figure 2.

Chromatogram with the spots: standard_Pg_standard

The overview of the chromatate-plate scanned at 206 nm showed an obvious peak both for standards and samples at Rf 0.9 (Figure 3).

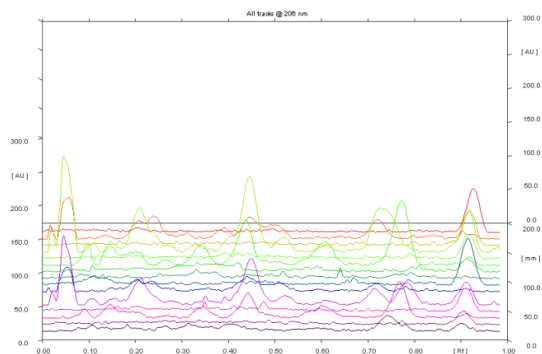


Figure 3.

Overall densitogram with standard solution and samples of different concentrations

The purity of the spectra for the bands in the samples attributed to β -sitosterol was confirmed using the WinCATS software by comparing the spectra on the ascendant slope, the middle and the descendent slope of each spot belonging to phytosterols to the ones belonging to the standard. As the three spectra are not very similar one to another, one can conclude with reasonable certainty there are no coelutions in the spot attributed to β -sitosterol (or if such a coelution exists, it does not affect the quantitative measurements).

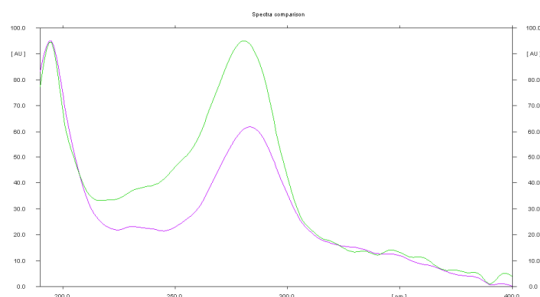


Figure 4.

Spectra comparison between the standard (green) and *P. grandiflora* (purple)

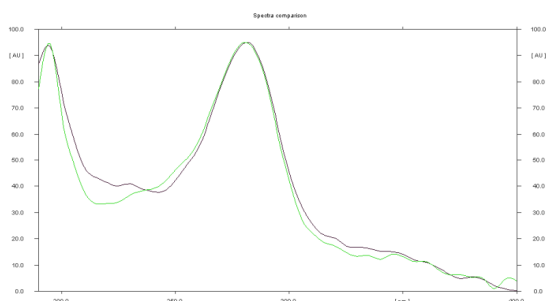


Figure 5.

Spectra comparison between the standard (green) and *P. oleracea* (purple)

By over posing the absorption spectra for the standards and for each sample, we found that in the

UV range the spectra are similar; this fact confirms that in both samples, of *P. oleracea* and of *P. grandiflora*, the β -sitosterol is present (Figure 4-5). The sterol quantity in each sample was calculated based on the calibration curve in Figure 6, using β -sitosterol as a standard. The calibration curve was drawn using samples from the stock solution of β -sitosterol with known concentrations.

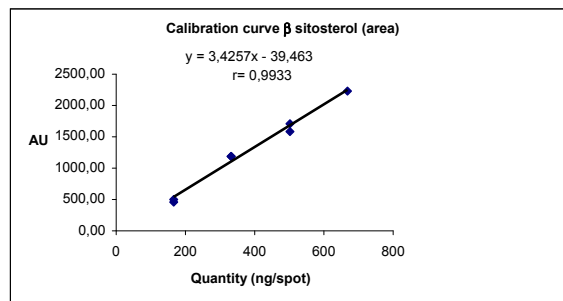


Figure 6.

Calibration curve for β -sitosterol

The linearity range investigated was 167-667 ng/mL. The method was validated in accordance with the International Conference on Harmonization (ICH) regulations, in terms of accuracy, limit of detection (LOD) and quantification (LOQ) (Table II) [17].

Table II

Validation parameters

Parameter	Value
Accuracy	99.06 $\pm$ 7.88
Slope	3.4257
Intercept	39.463
Linearity range	167-667 ng/mL
Correlation coefficient r	0.9933
SE of intercept	81.23
SD of intercept	229.87
LOD	201.31 ng/mL
LOQ	671.02 ng/mL

The concentrations of phytosterol from the samples were calculated based on the calibration curve, using the following formula:

$$\% \beta\text{-sitosterol} = C/\text{Mhp} * V_1/V_2 * 100$$

where C – concentration of β -sitosterol determined from the curve (ng); Mhp – mass of herbal product used (g); V_1 – solution volume obtained from the herbal product (mL); V_2 – spotted sample volume (μ L). The results are presented in Table III.

Table III

Sterol amount in the two assayed samples: *P. oleracea* and *P. grandiflora*

No.	Sample	Mean (mg sterol/100 g herbal product)	SD
1	<i>P. oleracea</i>	73.1 $\pm$	17.7
2	<i>P. grandiflora</i>	79.9 $\pm$	72.6

Mean = average of the calculated sterol concentrations; SD = standard deviation

Conclusions

Using high performance thin layer chromatography (HPTLC) we have confirmed the presence of β sitosterol in the methanolic extracts of herb from *P. oleracea* and *P. grandiflora* and we performed a semiquantitative assay of this component. While for *P. oleracea* there are previous reports regarding the sterols, for *P. grandiflora* no such qualitative or quantitative data on sterols could be found in the available and consulted literature.

The analysis of the methanolic extract through HPTLC revealed the presence of sterols in both plants. The semiquantitative assay indicated a β -sitosterol content of 79.9 mg per 100 g of herbal product for *P. grandiflora* and of 73.1 mg per 100 g of herbal product for *P. oleracea*, expressed on a dried basis.

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