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

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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 therapeutic 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 Rₑ 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 methanic 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
Steroli sunt metaboliti 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 qualitative și cantitative a sterolilor din Portulaca oleracea L. și Portulaca grandiflora Hook., în vederea potențialei lor valorificări terapeutic. S-au obținut extracte alcoolice din părurile aeriene ale Portulaca grandiflora și Portulaca oleracea, și s-a aplicat analiza HPTLC acestor extracte, utilizând plăci cu silicagel 60 F₂₅₄ și chloroform-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 Rₑ 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 cyclo-pentanoperhydro-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].
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 µL 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 µL and 20 µL. 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 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 Rf values and the results are being presented in Table I.

Table I

<table>
<thead>
<tr>
<th>No.</th>
<th>Standard/sample</th>
<th>Mean Rf</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard- β sitosterol</td>
<td>0.9077</td>
<td>0.0117</td>
</tr>
<tr>
<td>2</td>
<td>P. oleracea (Po)</td>
<td>0.9025</td>
<td>0.0050</td>
</tr>
<tr>
<td>3</td>
<td>P. grandiflora (Pg)</td>
<td>0.9075</td>
<td>0.0096</td>
</tr>
</tbody>
</table>

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

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 Rf values and the results are being presented in Table I.
The overview of the chromate-plate scanned at 206 nm showed an obvious peak both for standards and samples at Rf 0.9 (Figure 3).

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).

By over posing the absorbtion 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.

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].

![Figure 3. Overall densitogram with standard solution and samples of different concentrations](image1)

![Figure 4. Spectra comparison between the standard (green) and *P. grandiflora* (purple)](image2)

![Figure 5. Spectra comparison between the standard (green) and *P. oleracea* (purple)](image3)

![Figure 6. Calibration curve for β-sitosterol](image4)

![Calibration curve β-sitosterol (area)](image5)

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

\[
\% \beta\text{-sitosterol} = \frac{C}{\text{Mhp}} \times \frac{V_1}{V_2} \times 100
\]

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 (\(\mu\)L).

The results are presented in Table III.

### Table II

<table>
<thead>
<tr>
<th>Validation parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>99.06 ± 7.88</td>
</tr>
<tr>
<td>Slope</td>
<td>3.4257</td>
</tr>
<tr>
<td>Intercept</td>
<td>39.463</td>
</tr>
<tr>
<td>Linearity range</td>
<td>167-667 ng/mL</td>
</tr>
<tr>
<td>Correlation coefficient r</td>
<td>0.9933</td>
</tr>
<tr>
<td>SE of intercept</td>
<td>81.23</td>
</tr>
<tr>
<td>SD of intercept</td>
<td>229.87</td>
</tr>
<tr>
<td>LOD</td>
<td>201.31 ng/mL</td>
</tr>
<tr>
<td>LOQ</td>
<td>671.02 ng/mL</td>
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</tbody>
</table>

### Table III

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Mean (mg sterol/100 g herbal product)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>P. oleracea</em></td>
<td>73.1 ± 17.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>P. grandiflora</em></td>
<td>79.9 ± 72.6</td>
<td></td>
</tr>
</tbody>
</table>

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.

References