Quantitative determination of α-tocopherol and quality control studies in Sarcopoterium spinosum L.

Buket Bozkurt Sankaya, Hüsniye Kayalar

ABSTRACT: A quantitative determination of α-tocopherol in Sarcopoterium spinosum L. extracts was carried out by TLC-densitometry and HPLC-UV method. The α-tocopherol content in S. spinosum was established between 0.017210-0.023744 % (TLC-densitometry) and 0.025966-0.037212 % (HPLC-UV). The highest amount of α-tocopherol was obtained from aerial parts in fruiting period in both methods. In addition, contents of humidity, total ash and sulphated ash of plant samples were determined according to DAB 10.

KEY WORDS: Sarcopoterium spinosum, α-Tocopherol, TLC-densitometry, HPLC-UV

INTRODUCTION

Sarcopoterium spinosum L. (Rosaceae) is low, mound-forming spiny common shrub in the Eastern Mediterranean countries (1). In folk medicine, S. spinosum is reported to have antidiabetic effect (2, 3). In the literature there are several reports about the antidiabetic activity of S. spinosum (4-8).

Vitamine E has eight naturally occuring stereoisomers, four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ) (9). Among these compounds, most studies on vitamine E are carried out with α-tocopherol. Vitamine E has been shown to possess several biological properties, such as antioxidant, cancer-preventive effect (10). In addition, it could decrease the risk of the heart damage to the oxidative stress (11).

For the quantitative determination of α-tocopherol; spectrophotometric (12), HPLC (13), TLC (14) and GC-MS (15) methods have been suggested. In this study, aerial and underground parts of S. spinosum in two vegetation periods have been used to determine α-tocopherol content by performing modified TLC and HPLC-UV methods. The results of two different analytical methods have been compared in this text. Furthermore, contents of humidity, ash and sulphated ash of drug specimens were performed in accordance with DAB 10 (16) for the purpose of obtaining data for expectative monographs.

MATERIALS AND METHODS

Plant Material
The aerial and underground parts of S. spinosum were collected from Seferihisar, Izmir during both flowering and fruiting periods. The plant was identified by Prof. M. Ali Onur from the Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Izmir (Turkey). A voucher specimen (No. 1323) is deposited in the Herbarium of the Faculty of Pharmacy, Department of Pharmacognosy, Ege University.

Humidity, Total Ash, Sulphated Ash Determinations
Quality control determinations on plant materials (humidity, total ash and sulphated ash) were conducted according to methods in DAB 10. Aerial and underground parts of S. spinosum in flowering and fruiting periods were individually studied to the determine the quality control of plant.

Extraction
Four different extracts were prepared from the specimens of S. spinosum: A (aerial parts, flowering), B (aerial parts, fruiting), C (underground parts, flowering) and D (underground parts, fruiting). 100 g of each samples of S. spinosum were extracted with n-hexane (1 x 600 ml first for 5 h and than 2 x 600 ml for 8h) under stirring and filtered. The extraction solvent was evaporated in vacuo at 40ºC.
dl-α-Tocopherol (Roche) was used as a standard. The n-hexane used for the extraction was obtained from Merck, whereas the methanol used as eluent in the high-performance liquid chromatography (HPLC) system was purchased from LabScan. Other solvents and reagents were obtained from Merck.

**Sample Solutions**

10 mg of each extract was dissolved in 2.5 ml methanol for HPLC-UV method and 20-60 mg extract in 2 ml chloroform for TLC densitometric assay.

**TLC-densitometric assay**

A Shimadzu high-speed TLC scanner CS-920 was used with the following settings: beam size of 0.4 X 0.4 mm, X=24, Y=10, L=3; AZS off, wavelength of 350 nm. Silica gel 60F 254 (20 X 20 cm, 0.25 mm thick, Merck) plates were used. Cyclohexane/diethylether (4:1) was used as mobile phase. The samples were implemented with Hamilton syringes (15 mm from the bottom line of the plate). The mobile phase was allowed to run a distance of 100 mm in the saturated tank.

Silica plates were prewashed in chloroform/methanol (1:1), dried and activated at 100°C for 10 min. α-Tocopherol solutions (2, 4, 6 and 8 μl) were applied on a TLC plate and developed under the same conditions. The developed plates were initially air-dried and then oven-dried for 15 min at 100°C, and sprayed with CuSO₄-phosphoric acid reagent (10% CuSO₄ / 8% phosphoric acid, 1:1) followed by charring at 190°C for 10 min. The α-tocopherol quantity of the samples were measured by Thin Layer Scanner at 350 nm using a D₂ lamp. The calibration curve exhibited a linear relationship between the quantities and areas on TLC plates (Fig. 1). 20 μl of sample solutions were applied on TLC plate and after the development the areas of the spots were integrated by TLC-densitometry. Each analysis was carried out in triplicate.

**High-Pressure Liquid Chromatography-UV Method**

The HPLC system (Hewlett Packard 1100 series) equipped with a UV variable-wavelength detector (HP 1100) set at 292 nm. A highrom 5 C18 column (25 cm x 4.6 mm i.d.) was eluted with methanol at a flow rate of 2 ml/min. A manual injector with 20 μl loop (HP 1100 G1328A Rheodyne 7725i) and the column temperature was adjusted to 40°C.

For the preparation of the calibration curve of α-tocopherol, 2 mg of the standard was dissolved in 1 ml methanol. 0.5, 1, 2, 4, 5, 10, 15, 20 and 25 μg/20 μl concentrations were prepared from stock solution. Twenty microliters of the standard solutions were injected on the HPLC column. Then, the calibration curve of α-tocopherol was drawn (Fig. 2). 10 mg of extracts were dissolved in methanol (2.5 ml). Each aliquot was injected into the HPLC column with a volume of 10 μl. For each sample, the procedure was repeated three times.

**RESULTS**

**Quality Control Studies**

Quality control determinations (humidity, total ash and sulphated ash) on drug specimens prepared separately from plants in flowering and fruiting periods were conducted according to methods in DAB 10.

The results of the assays (Table 1) provide knowledge for the prospective monographs of Herba and Radix Poterii spinosi.

**TABLE 1. Quality Control Determinations on Herba and Radix Poterii spinosi prepared from Sarcopoterium spinosum**

<table>
<thead>
<tr>
<th>Drug Specimen</th>
<th>Humidity (%)</th>
<th>Total Ash (%)</th>
<th>Sulphated Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial parts, flowering period</td>
<td>5.4275 ± 0.0364</td>
<td>4.9621 ± 0.1444</td>
<td>7.2186 ± 0.1952</td>
</tr>
<tr>
<td>Aerial parts, fruiting period</td>
<td>5.338 ± 0.0104</td>
<td>5.2358 ± 0.0619</td>
<td>7.6126 ± 0.0313</td>
</tr>
<tr>
<td>Underground parts, flowering period</td>
<td>3.050 ± 0.0112</td>
<td>9.9642 ± 0.0790</td>
<td>13.0257 ± 0.0295</td>
</tr>
<tr>
<td>Underground parts, fruiting period</td>
<td>3.0354 ± 0.0067</td>
<td>10.0206 ± 0.0293</td>
<td>14.0974 ± 0.0124</td>
</tr>
</tbody>
</table>

*Mean Results ± Standard Deviations*
TABLE 2. Contents of α-Tocopherol (% on dry wt.) in S. spinosum as determined by TLC-Densitometry and HPLC-UV Method

<table>
<thead>
<tr>
<th>Sample</th>
<th>TLC-densitometry#</th>
<th>HPLC-UV#</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>0.017210 ± 0.00058</td>
<td>0.025966 ± 0.00035</td>
</tr>
<tr>
<td>(B)</td>
<td>0.023744 ± 0.00039</td>
<td>0.037212 ± 0.00041</td>
</tr>
</tbody>
</table>

# Mean Results ± Standard Deviations

**TLC-Densitometric Assay**

α-Tocopherol content of Sarcopoterium spinosum extracts was quantitatively determined by TLC-densitometric method. Each extracts and standard compound were tested for three times. The results of our studies revealed that α-tocopherol is not present in underground parts of S. spinosum collected during both flowering and fruiting seasons (Sample C and D). For the calculation of the α-tocopherol content of extracts, TLC-densitometric curve and following linear equation were used.

\[ y = 1576.5x + 1177.9 \]  \( R^2 = 0.9995 \)

where \( x \) is the α-tocopherol concentration (µg/ml) and \( y \) is the area (integration unit). The results of the assay are shown in Table 2.

**HPLC-UV Method**

The identification and quantitative determination of α-tocopherol in the extracts were carried out by a comparison of retention times and areas with that of standard α-tocopherol. The results showed that α-tocopherol is not present in sample C and D. The α-tocopherol content in the 9-hexane extracts of the aerial parts of the S. spinosum was calculated from the following regression equation of the calibration curve:

\[ y = 198.13x + 3.4285 \]  \( R^2 = 0.9992 \)

where \( x \) is the α-tocopherol concentration (µg/ml) and \( y \) is the peak area. The results of the assay are shown in Table 2.

**DISCUSSION**

To the best of our knowledge, the quality control and α-tocopherol content of S. spinosum was studied for the first time in this study. The results of the humidity, total ash and sulphated ash assays will be convenient to prospective monographs on Herba and Radix Poterii spinosi (Table 1). The quantitative determination of α-tocopherol in S. spinosum extracts was accomplished by a comparison standard α-tocopherol. The α-tocopherol amount of aerial parts was determined between 0.017210 % (sample A)-0.023744 % (sample B) by TLC-densitometry and 0.025966 % (sample A)-0.037212 % (sample B) by HPLC-UV method (Table 2). Our results showed that HPLC-UV assay exhibited higher amounts of α-tocopherol because of the sensibility of the method. The highest rates were detected in the sample B (aerial parts, fruiting period) in both method. According to results, α-tocopherol is not present in underground parts of the plant (sample C and D). The α-tocopherol quantity may be used as a criterion for the standardization and evaluation of the quality of S. spinosum for therapeutic or supportive utilization.

Soya bean oil is the most important industrial source of α-tocopherol. However, the α-tocopherol content in this source is only 0.0051-0.0111% (17). Moreover S. spinosum has up to five-times higher amount of α-tocopherol than soya bean. In light of this, this plant should be considered as a potential new source of α-tocopherol.

On the other hand, there is evidence that plasma α-tocopherol concentrations are lower in diabetics (18). Last studies have shown that vitamine E could protect kidney, pancreas, eye and nervous system against the undesirable effects of diabet in animals (19). These datas could support the use of S. spinosum for diabetes in folk medicine.

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**REFERENCES**


