 DOI: 10.12991/201115438

ORIGINAL RESEARCH

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

Buket Bozkurt Sarıkaya, 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

AFFILIATIONS
Department of Pharmacognosy, Faculty of Pharmacy, Ege University, 35100, Bornova, Izmir, Turkey

CORRESPONDENCE
Buket Bozkurt Sarıkaya
E-mail:
buket.bozkurt@ege.edu.tr

Received:
03.11.2010
Revision:
22.11.2010
Accepted:
01.12.2010

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 40^0C.

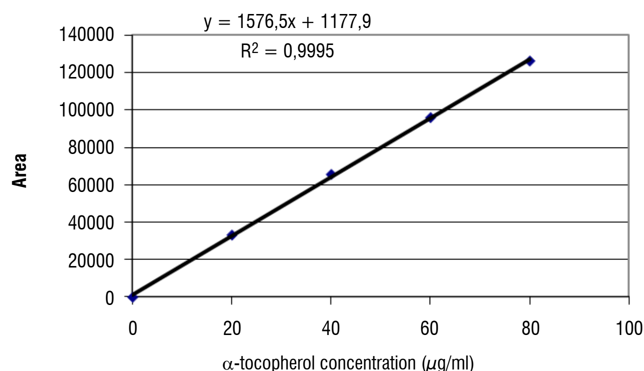

FIGURE 1. Calibration Curve for the Determination of α-Tocopherol by TLC-Densitometry

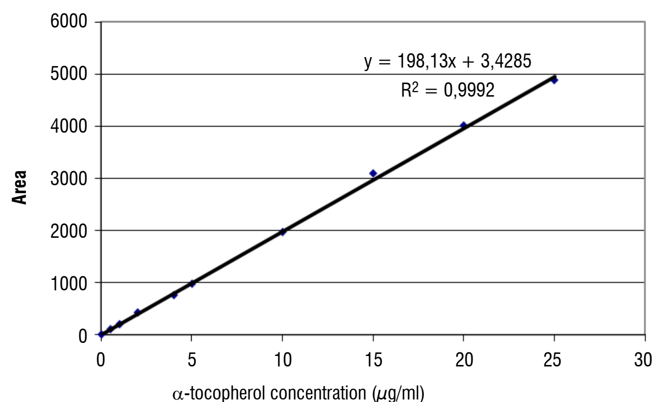

FIGURE 2. The Calibration Curve for the HPLC-UV Determination of α-Tocopherol

Chemicals

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 Lab-Scan. 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 100^0C 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 100^0C, and sprayed with $CuSO_4$-phosphoric acid reagent (10% $CuSO_4$ / 8% phosphoric acid, 1:1) followed by charring at 190^0C for 10 min. The α-tocopherol quantity of the samples were measured by Thin Layer Scanner at 350 nm using a D_2 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 hichrom 5 C_{18} 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 40^0C.

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/20µl 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*

Drug Specimen	Humidity (%)*	Total Ash (%)*	Sulphated Ash (%)*
Aerial parts, flowering period	5.4275 ± 0.0364	4.9621 ± 0.1444	7.2186 ± 0.1952
Aerial parts, fruiting period	5.3388 ± 0.0104	5.2358 ± 0.0619	7.6126 ± 0.0313
Underground parts, flowering period	3.0500 ± 0.0112	9.9642 ± 0.0790	13.0257 ± 0.0295
Underground parts, fruiting period	3.0354 ± 0.0067	10.0206 ± 0.0293	14.0974 ± 0.0124

*Mean Results ± Standard Deviations

TABLE 2. Contents of α-Tocopherol (% on dried wt.) in *S. spinosum* as determined by TLC-Densitometry and HPLC-UV Method

Sample	TLC-densitometry#	HPLC-UV#
(A)	0.017210 ± 0.00058	0.025966 ± 0.00035
(B)	0.023744 ± 0.00039	0.037212 ± 0.00041

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.

$$y = 1576.5\,x + 1177.9; \quad R^2 = 0.9995$$

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 *n*-hexane extracts of the aerial parts of the *S. spinosum* was calculated from the following regression equation of the calibration curve:

$$y = 198.13\,x + 3.4285; \quad R^2 = 0.9992$$

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.

ACKNOWLEDGEMENTS

This work was supported by the Research Fund of Ege University (Project No: 04/ECZ/008).

REFERENCES

1. Cullen J. Flora of Turkey and the East Aegean Islands, Ed. Davis P. H. Universiy Press, Edinburg, 1972, vol. 4, pp. 76-77.
2. Baytop T. Türkiye'de Bitkilerle Tedavi Geçmişte ve Bugün, İstanbul Üniversitesi Eczacılık Fakültesi Yayınları, No: 3255:167, 1984.
3. Yaniv Z, Dafni S, Friedman J, Palevitch D. Plants used for the treatment of diabetes in Israel. J Ethnopharmacol, 19: 145–51, 1987.
4. Menzcel E. Hypoglycemic substances. Exp Med, 102: 253-256, 1963.
5. Schluetz GO, Venulet J. Poterium spinosum and its effect on the blood sugar contents of rabbits, Experientia, 20: 78-79, 1964.
6. Mishkinsky J, Menzcel E, Sulman FG. Hypoglycemic effect of Poterium spinosum. Arch Int Pharmacod T, 161: 306-313, 1966.

Sarcopoterium spinosum L. Üzerinde α-Tokoferol Miktar Tayini ve Kalite Kontrol Çalışmaları

ÖZET: *Sarcopoterium spinosum* L. ekstrelerinde α-tokoferol miktar tayini TLC-dansitometri ve HPLC-UV yöntemleriyle araştırılmıştır. *S. spinosum*'un α-tokoferol içeriği 0.017210-0.023744 % (TLC-dansitometri) ve 0.025966-0.037212 % (HPLC-UV) aralığında tespit edilmiştir. En yüksek α-tokoferol miktarı, her iki yöntemde de meyveli dönem toprak üstü kısımlarından elde edilmiştir. Bunlara ek olarak, bitki örneklerinin nem içeriği, total kül ve sülfat külü miktarı DAB 10'a göre tayin edilmiştir.

ANAHTAR KELİMELER: *Sarcopoterium spinosum*, α-Tokoferol, TLC-dansitometri, HPLC-UV

7. Slijepcevic M, Kraus L. The diabetic mouse as an experimental for measuring the blood glucose-lowering effects of plant extracts and insulin preparations. Acta Therap, 23: 47–58, 1997.
8. Smirin P, Taler D, Abitbol G, Brutman-Barazani T, Kerem Z. Sarcopoterium spinosum extract as an antidiabetic agent: in vitro and in vivo study. J Ethnopharmacol, 129: 10-17, 2010.
9. Huang H-Y, Appel LJ. Supplementation of diets with α-tocopherol reduces serum concentrations of γ- and δ-tocopherol in humans. J Nutr, 133: 3137-3140, 2003.
10. Ju J, Picinich SC, Yang Z, Suh N, Kong A-N, Yang CS. Cancer-preventive activities of tocopherols and tocotrienols. Carcinogenesis, 31: 533-542, 2010.
11. Rodrigo R, Vinay J, Castillo R et al. Use of vitamins C and E as a prophylactic therapy to prevent postoperative atrial fibrillation. Int J Cardiol, 138: 221-228, 2010.
12. Booth VH. α-Tocopherol, its co-occurrance with chlorophyll in chloroplast. Phytochemistry, 2: 421-427, 1963.
13. Gapor A, Kato A, Ong ASH. Tocopherol content in oil palm leaflet. J Am Oil Chem Soc 63: 330-331, 1986.
14. Chevolleau S, Mallet JF, Debal A, Ucciani E. Antioxidant activity of mediterranean plant leaves: occurence and antioxidative importance of α-tocopherol. J Am Oil Chem Soc, 70: 807-809, 1993.
15. Barbosa LCA, Teixeira RR, Demuner AJ, Madruga MS. Vitamine E and other chemical constituents of the leaves of Gallesia gorazema. Fitototerapia, 68: 515-519, 1997.
16. Deutsches Arzneibuch 10. 1997.
17. Slover HT, Thompson RH, Merola GV. Determinations of tocopherols and sterols by capillary gas chromatography. J Am Oil Chem Soc, 60: 1524-1528, 1983.
18. Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritschler H, Rosen P, Halliwell B, Betteridge DJ. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. Diabetologia, 40: 647-653, 1997.
19. Pazdro R, Burgess JR. The role of vitamin E and oxidative stress in diabetes complications. Mech Ageing Dev, 131: 276-286, 2010.