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Fitoterapia 76 (2005) 62–66

FITOTERAPIA

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Quantitative determination of α-tocopherol in *Pistacia lentiscus*, *Pistacia lentiscus* var. *chia*, and *Pistacia terebinthus* by TLC-densitometry and colorimetry

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Received 20 January 2003; accepted in revised form 24 September 2004
Available online 8 December 2004

Abstract

A quantitative determination of α-tocopherol in *Pistacia lentiscus*, *Pistacia lentiscus* var. *chia*, and *Pistacia terebinthus*, leaves was established by TLC-densitometry and colorimetry. The highest amount of α-tocopherol was found in *P. lentiscus* var. *chia*.

Keywords: *Pistacia lentiscus*; *Pistacia lentiscus* var. *chia*; *Pistacia terebinthus*; α-Tocopherol; TLC-densitometry; Colorimetry

1. Introduction

The leaves of *Pistacia lentiscus* L.(Anacardiaceae) are extensively used in folk medicine for the treatment eczema, diarrhoea, and throat infections, and as a potent antiulcer agent [1]. The aerial parts of this species have traditionally been used in Mediterranean area as a popular cure for hypertension [2]. The resin obtained from *Pistacia lentiscus* var. *chia* is gum mastic or masticha. Gum mastic is used in cosmetics

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doi:10.1016/j.fitote.2004.09.021

and perfumery, as a flavouring in food technology and for its antimicrobial activity especially against *Helicobacter pylorii* [3]. *Pistacia terebinthus* L., commonly known as melengic, is extensively used in Turkish folk medicine for the treatment of sunstroke, peptic ulcer, and asthma [4,5].

α-Tocopherol (vitamin E) is naturally occurring in *Pistacia* leaves. The pharmacological properties of this vitamin, widely used as a natural antioxidant, are well known [6–8]. Moreover, α-tocopherol was used in cosmetology [9].

For the quantitative determination of α-tocopherol, spectrophotometric [10,11], HPLC [12,13], TLC [13,14], and GC–MS [15] have been suggested. In this study, modified TLC-densitometric and colorimetric methods have been used to determine the α-tocopherol content in *P. lentiscus*, *P. lentiscus* var. *chia*, and in *P. terebinthus*. The results obtained from the two analytical methods have been compared.

2. Experimental

2.1. Plant material

P. terebinthus leaves were collected from West Anatolia in Izmir-Buca, in May 2001. *P. lentiscus* var. *chia* and *P. lentiscus* leaves were collected from West Anatolia in Izmir-Çesme, in February 2001 and identified by B. KIVÇAK. Voucher specimens (No. 1269, 1268, 1267) are deposited in the Herbarium of the Faculty of Pharmacy, Ege University in Izmir.

2.2. Extraction

A sample (100 g) of accurately weighed, air-dried, and powdered leaves was extracted with *n*-hexane (2×600 ml first for 5 h and then for 8 h) under stirring. The combined organic phases were filtered and distilled in vacuo to yield the extract, which was stored at −20 °C [14]. The percentage yields of extracts are indicated in Table 1.

2.3. Reagents and solvents

α-Tocopherol (Sigma) was used as standard. All of the analytical grade solvents and reagents were purchased from Merck.

Table 1
Contents of the α-tocopherol (percent (%) on dried wt.) in *Pistacia lentiscus* var. *chia* and *Pistacia terebinthus* leaves as determined by TLC densitometry and colorimetry[a]

Samples	Extract yields (%)	TLC-densitometry	Colorimetry
Pistacia lentiscus	5.2124	0.004345±0.00026	0.004334±0.00010
Pistacia lentiscus var. *chia*	5.8030	0.005211±0.00023	0.005308±0.00011
Pistacia terebinthus	5.1379	0.003299±0.00017	0.003374±0.00008

[a] Values are mean±S.E. (N=12); t_{11} (value of distribution with df=11): 0.975.

2.4. Sample solutions

Sample solutions were prepared by dissolving in $CHCl_3$ 20–60 mg (TLC densitometric assay) or 10-40 mg (colorimetric assay) of accurately weighed extract in a 10-ml volumetric flask.

2.5. TLC-densitometric assay

A Shimadzu high-speed TLC-Scanner CS-920 was used with the following settings: beam size of 0.4×0.4 mm, *X*=24,*Y*=10, *L*=3; AZS off, wavelength of 350 nm. Silica gel $60F_{254}$ (20×20 cm, 0.25-mm thick, Merck) plates were used. The mobile phase was cyclohexane/diethylether 4:1. Samples were applied with Hamilton syringes (15 mm from the bottom edge of the plate). The mobile phase was allowed to run a distance of 100 mm in the saturated tank.

Silica plates were prewashed in $CHCl_3$/MeOH 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 above-mentioned conditions. The developed plates were initially air-dried, then oven-dried for 15 min at 100 °C, and sprayed with 10% $CuSO_4$-phosphoric acid followed by charring at 190 °C for exactly 10 min. The resolved compounds were quantitated on the high-speed TLC scanner at 350 nm using a D_2 lamp. The calibration curve (Fig. 1) showed a linear relationship between the concentrations and areas on TLC plates. Aliquots (10 or 20 μl) of sample solutions (see Section 2.4) were spotted on each TLC plate, and after the development, the areas of the spot on the plate were integrated by TLC-densitometry. For every sample, the procedure was repeated three times.

2.6. Colorimetric assay

2.6.1. 2,2′-Dipyridyl reagent

2,2′-Dipyridyl (0.125 g) is dissolved in 25 ml of absolute EtOH, then stored in a dark bottle in the refrigerator until use.

2.6.2. Ferric chloride reagent

Ferric chloride hexahydrate (0.2 g) is dissolved in 100 ml of absolute EtOH. The solution is kept in the refrigerator until use.

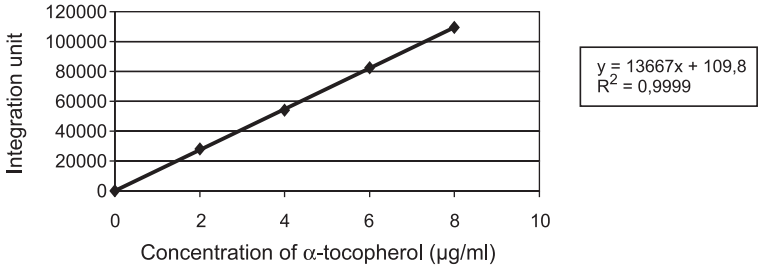

Fig. 1. Calibration curve for the determination of α-tocopherol by TLC-densitometry.

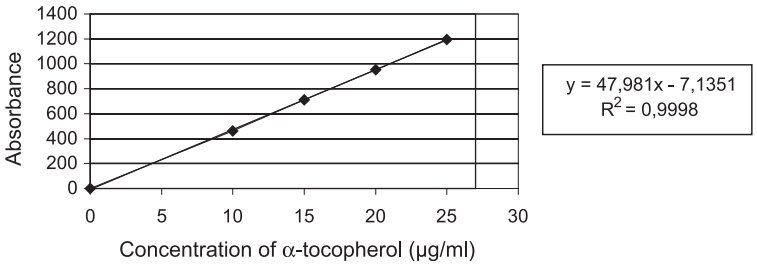

Fig. 2. Calibration curve for the colorimetric determination of α-tocopherol.

2.6.3. Procedure

A Shimadzu UV-160A model spectrophotometer was used. Aliquots (0.010, 0.015, 0.020, and 0.025 μl) of a 10 g/l solution of α-tocopherol in $CHCl_3$ were transferred to a volumetric flask and the volume was adjusted to 8 ml with $CHCl_3$. Each of the solutions and 1 ml of 2,2′-dipyridyl reagent were pipetted into a 10-ml volumetric flask and mixed. A 1-ml portion of ferric chloride reagent was added to the 10-ml volumetric flask and the mixture was shaken for 10 s. The absorbance of the mixture was read at 522 nm in a 1-cm cell 50 s after adding the ferric chloride. A blank was run, using 8 ml of $CHCl_3$, 1 ml of 2,2′-dipyridly reagent, and 1 ml of ferric chloride reagent. The absorbance of this solution was measured at 522 nm against a blank. Then, the standard curve was drawn (Fig. 2). The above-described procedure was followed by using 10, 20, 30, 40 mg sample solutions (see Section 2.4). The α-tocopherol content in the extracts was calculated from the regression equation of the standard curve.

2.7. Statistical analysis

Results obtained from TLC-densitometric and colorimetric analyses were expressed as mean±S.E. and compared by paired *t*-test ($P<0.05$).

3. Results and discussion

α-Tocopherol in *P. lentiscus*, *P. lentiscus* var. *chia*, and in *P. terebinthus* extracts were quantitatively determined by TLC-densitometry and colorimetry.

For testing the quantitative accuracy of the TLC-densitometric method, the analyses of the reference substance and of the extracts were repeated on three different plates. Excellent linearity was observed between concentrations and areas integrated by TLC densitometry. The TLC-densitometric calibration curve (Fig. 1), used to calculate the α-tocopherol content in the extracts, was expressed by the following linear equation:

$$y = 13667x + 109.8; \quad R^2 = 0.999$$

where y is the integration unit and x is the α-tocopherol concentration (μg/ml).

For the colorimetric assay of α-tocopherol, a calibration curve was prepared (Fig. 2), expressed by following linear equation:

$$y = 47.981 + 7.1351; \quad R^2 = 0.999$$

where y is the absorbance and x is the α-tocopherol concentration (μg/ml).

The results of quantitative determinations of α-tocopherol in *P. lentiscus*, *P. lentiscus* var. *chia*, in and *P. terebinthus* leaves by TLC-densitometry and colorimetry are shown in Table 1. Comparison of the TLC-densitometric results with those obtained from colorimetric experiments showed no significant differences, confirming the reliability of the colorimetric data.

The maximum content of α-tocopherol was found in the leaves of *P. lentiscus* var. *chia* (0.005308%) by colorimetry. The major industrial source of α-tocopherol is a residue obtained from the distillation of soya bean oil. Although this distillate contains 1.7–3.3 % of α-tocopherol, its content in the soya bean is only 0.051–0.0111% [16]. So this plant may be considered as a potential new source of this compound.

Acknowledgements

This research was supported by Ege University Research Fund (No.2001 /ECZ/ 009) Izmir, Turkey.

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