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DETERMINATION OF DIOSGENIN IN Tribulus terrestris L. GROWING IN TURKEY BY HPLC

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TÜRKİYE'DE YETİŞEN Tribulus terrestris L. BİTKİSİNDE YBSK YÖNTEMİ İLE DİOSGENİN MİKTAR TAYİNİ

ÖZET

Bu çalışmada, Türkiye'de yetişen Tribulus terrestris L. (Zygophyllaceae) bitkisinin kök, gövde+yapraklar ve meyvalarında YBSK yöntemi ile % 0.13, 0.78 ve 0.12 oranında diosgenin bulunduğu saptanmıştır.

SUMMARY

In this study, diosgenin contents of the roots, stem+leaves and fruits of Tribulus terrestris L. (Zygophyllaceae) growing in Turkey was determined to be 0.13, 0.78 and 0.12 %, respectively by HPLC.

Key Words: *Diosgenin, Tribulus terrestris, HPLC.*

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INTRODUCTION

Steroidal sapogenins especially diosgenin are used as starting material for the manufacture of medicinal steriods. Diosgenin is obtained commercially from the tubers of several Dioscorea species. New sources of diosgenin are being developed for commercial exploitation.

Tribulus terrestris is an annual plant distributed in the South Europe and warm and tropical countries of Asia and Africa (1,2). T. terrestris is named as "Çobançökerten", "Deveçökerten", "Çarıkdikeni" and "Demirdikeni" in Turkish(3). This plant is used in folk medicine as diuretic, tonic and against kidney stones(3), hypertension and hypercholesterolemia in Turkey, against impotensy (4) in Bulgaria and for the treatment of various diseases (5) in India. The saponin mixture of T. terrestris has been reported that it is useful as an antisclerotic agent and decreases the plasma cholesterol level (6,7). It is observed that Tribestan, a pharmaceutical preparation developed from the above ground parts of T. terrestris, increased the libido and spermatogenesis and was effective against frigidity, infertility and climacteric disturbances (4). The ether extract of the fruits of T. terrestris produced diuresis and increased the creatinine renal clearance in the anesthetized dogs (8).

β-carbolin alkaloids, steroidal saponins, steroidal sapogenins and flavonoid glycosides have been reported in T. terrestris (5). Diosgenin content of this plant has been found about 0.15-0.35 % by using different methods (9-12). However, there have been no reports on T. terrestris growing in Turkey. In this study, diosgenin content of the different parts of T. terrestris, collected in the vicinity of Ankara, was determined by HPLC (13).

MATERIAL and METHODS

Plant Material

Tribulus terrestris used in this research was collected from Kazan (Ankara-Turkey). Herbarium specimens are preserved in "Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu (AEF No. 15215)", Ankara, Turkey.

Equipment

Sapogenin fractions, obtained from the plant materials, were examined by TLC on silica gel GF_{254} (Merck) plates using toluene: acetone (85:15) solvent system, 30 % H_2SO_4 and/or anisaldehyde-H_2SO_4 reagent for detection of sapogenins.

HPLC was carried out with Waters Liquid Chromatograph Model 600 consisted of Model 6000 A pump, U6K injector, μ–Porasil column (300 x 3.9 mm I.D.) and a R-401 differential refractometer connected to an omniscribe recorder. The mobile phase was petroleum ether: isopropanol (12:1), flow rate 0.8 ml/min., pressure 200 psi., temperature 20° C, attenuation 8x and chart speed 1 cm/min.

Sample Preparation

Dried and powdered plant material (500 mg) was hydrolyzed by heating for 3h under reflux with 2.5 N HCl. After cooling and filtering the mixture, the acid-insoluble residue was washed with water until the residue was free from acid. The washed residue was dried at 105°C and then extracted with petroleum ether (50-75°) in a Soxhlet for 5h. The solvent was evaporated under vacuum. Each residue (9.5 mg, 21.1 mg and 14.4 mg) obtained from roots, stem+leaves and fruits, respectively was dissolved in HPLC grade petroleum ether: isopropanol (12:1) and filtered from fluoropore 0.5 μ filter (Waters). The solution was then made up a suitable concentration by dilution with the same solvent system. A 20 μl volume of the solution was injected into the chromatograph with a 25 μl Hamilton syringe.

Preparation of Standards

A set of eight standard solutions were prepared containing 0.25-2.0 mg/ml of diosgenin (Sigma).

RESULTS and DISCUSSION

Diosgenin contents of the stem+leaves, roots and fruits of Tribulus terrestris were determined separately by HPLC. External standard method was used for quantitative determination. The amounts of the diosgenin present in the samples were calculated from comparisons of peak heights with the calibration graph (Fig. 1) constructed from the standard solutions. All compounds were eluted in 7 minutes. Chromatograms of the standards and samples are shown in Fig. 2-5. Each analysis was repeated five times. Results (on dry weight basis) are given in Table 1.

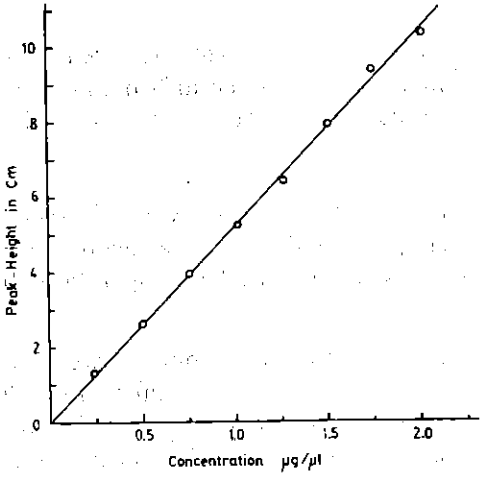

Fig. 1. Calibration graph for diosgenin.

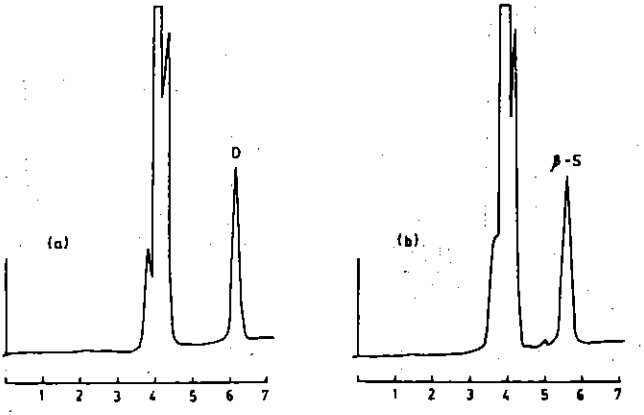

Fig. 2. HPLC chromatograms of a) diosgenin b) β-sitosterol

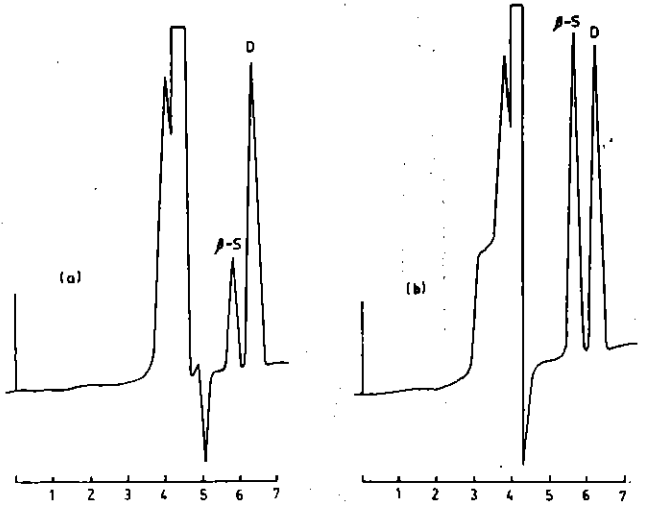

Fig . 3. HPLC chromatograms of a) stem+leaves
b) (stem+leaves) +β-sitosterol.

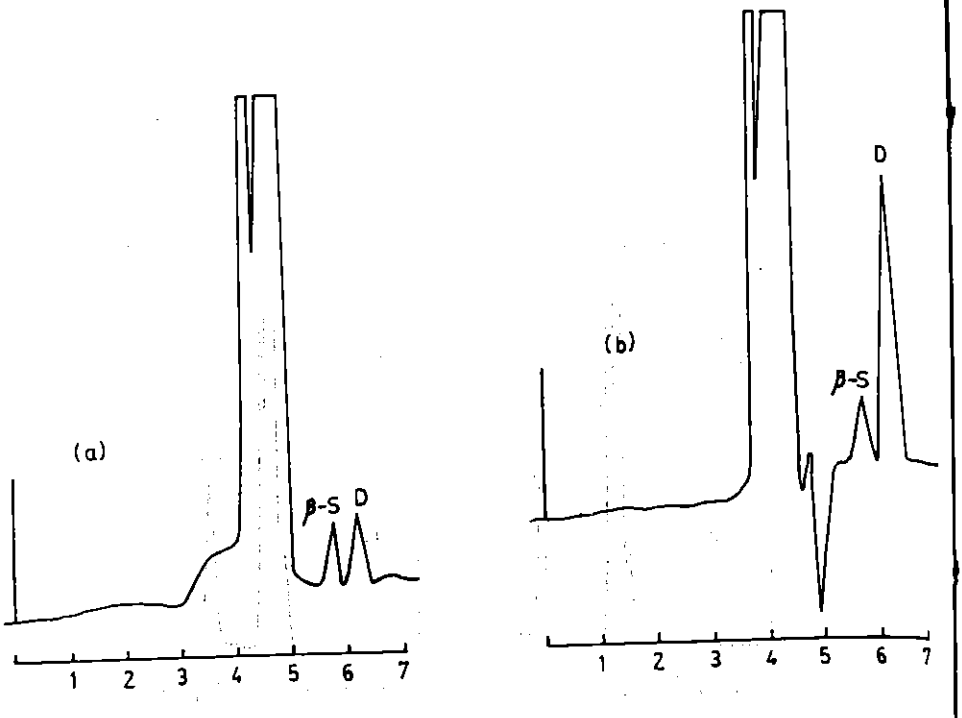

Fig. 4. HPLC chromatograms of a) fruits b) fruits+diosgenin.

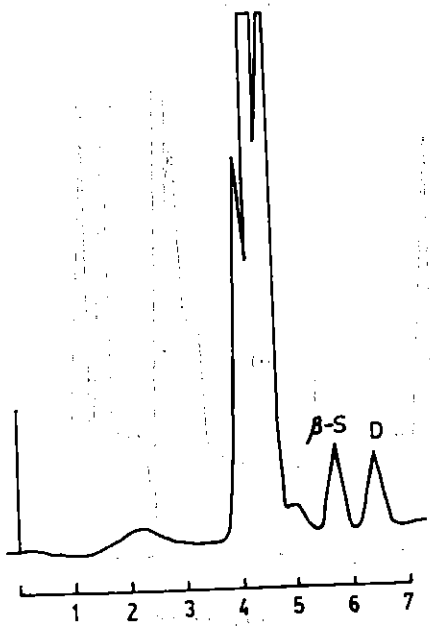

Fig. 5. HPLC chromatogram of roots.

Table 1. Diosgenin contents of studies samples

Samples	Diosgenin (%)
Stem+leaves	0.78
Fruits	0.12
Roots	0.13

In Tribulus terrestris, the highest diosgenin content (0.78 %) was found in the stem+leaves; roots and fruits showed 0.13 and 0.12%, respectively. The level of diosgenin in stem+leaves seems to be higher than those of T. terrestris growing in other countries (9-12).

β-Sitosterol was detected in addition to diosgenin in the studied samples by this method.Other constituents, observed in the crude sapogenin mixture by TLC, will be published after isolated and identified.

REFERENCES

1. Davis, P.H. (1966) Flora of Turkey and the East Aegean Islands, Vol. 2, p. 493, University Press, Edinburgh.
2. List, P.H. and Horhammer, L. (1979) Hager's Handbuch der Pharmazeutichen Praxis, Vol. 6 (3), p. 241, Springer-Verlag, Berlin.
3. Baytop, T. (1984) Türkiye'de Bitkiler ile Tedavi, p.402, Sanal Basımevi, Istanbul.
4. Tomova, M. (1987) Tribestan, Farmatsiya, 37 (6), 40-2. Ref. C.A., Vol. 108, 161409d, 1988.
5. Mahato, S.B., Sahu, N.P., Ganguly, A.N. (1981) Steroidal Glycosides of Tribulus terrestris Linn., J. Chem. Soc. Perkin Trans. 1, 9, 2405-10.
6. Umikashvili, R.S. (1972) Histochemical Characteristics of the Liver in Experimental Hypercholesterolemia under the Action of T. terrestris Saponins, Soobshch. Akad. Nauk.Gruz. SSR, 67 (3), 729-31. Ref. C.A., Vol. 78, 12203 s, 1973.
7. Kemertelidze, E.P., Pkheidze, T.A., Kachukhashvili, T.N., Turova, A.D., Sokolova, L.N., Umikashvili, R.S. (1977) "Tribusponin"-an antisclerotic agent, Otkrytiya Izobret. Prom. Obraztsy Tovarnye Znaki, 54 (29), 10. Ref. C.A., Vol. 87, 157181d, 1977.
8. Singh, R.C.P., Sisodia, C.S. (1971) Effect of Tribulus terrestris Fruits Extracts on Chloride and Creatinine Renal Clearances in Dogs, Indian J. Physiol. Pharmacol., 15 (3), 93-6.

9. Kachukhashvili, T.N. (1965) Diosgenin from Tribulus terrestris growing in Georgian S.S.R., Med. Prom. SSSR, 19 (3), 46-8. Ref. C.A., Vol. 63, 1654 d, 1965.

10. Gheorghiu, A., Ionescu-Matiu, E. (1968) Presence of Chlorogenin with Diosgenin and Gitogenin in Tribulus terrestris, Ann.Pharm. Fr., 26 (12), 745-48.

11. Abrosimov, S.P., Shchelochkova, A.P., Denikeeva, M.F., Koshoev, K.K. (1970) Kirghiz SSR Plants Containing Steroid Saponins, Fiziol. Aktiv. Soedin. Rast.Kirg., 59-63. Ref. C.A., Vol. 76, 83492 e, 1972.

12. Mahato, S.B., Sahu, N.P., Pal, B.C., Chakravarti, R.N., Chakravarti, D., Ghosh, A. (1978) Screening of Tribulus terrestris Plants for Diosgenin, J. Inst.Chem. (India), 50 (1), 49-50.

13. Mahato, S.B., Sahu, N.P., Roy, S.K. (1981)High-Performance Liquid Chromatographic Method for the Determination of Diosgenin in Plants, J. Chromatog., 206 (1), 169-73.