

Alkaloids of *Tribulus terrestris* L. Growing in Turkey

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Abstract: Alkaloids of *Tribulus terrestris* L. (Zygophyllaceae) were isolated by preparative TLC and identified as harman, harmine, harmaline and harmalol on the basis of their physical and spectral properties in this research. Titrimetric method was used to estimate the total alkaloid content.

Keywords : *Tribulus terrestris*, Zygophyllaceae, alkaloids, harman, harmine, harmaline, harmalol.

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Türkiye'de Yetişen *Tribulus terrestris* L. Bitkisinin Alkaloitleri

Özet: Bu araştırmada, *Tribulus terrestris* L. (Zygophyllaceae) bitkisinin alkaloitleri preparatif İTK ile izole edilmiş ve fiziksel ve spektral özelliklerinden yararlanılarak harman, harmin, harmalin ve harmalol olarak teşhis edilmiştir. Total alkaloit miktar tayini için titrimetrik yöntem kullanılmıştır.

Anahtar kelimeler : *Tribulus terrestris*, Zygophyllaceae, alkaloitler, harman, harmin, harmalin, harmalol.

Introduction

Tribulus terrestris L. (Zygophyllaceae) is a hairy prostrate herb distributed in South Europe and subtropic and tropical countries of Asia and Africa^{1,2}. *T. terrestris* is named as "Çobançökerten, Deveçökerten, Çarıkdikeni and Demirdikeni" in Turkish³. This plant is used in folk medicine as diuretic, tonic and to pass kidney stones³, hypertension and hypercholesterolemia in Turkey. It is used against impotency⁴ in Bulgaria and for the treatment of various diseases⁵ in India.

β-carbolin alkaloids, steroidal saponins, and flavonoid glycosides have been reported in *T. terrestris*^{5,6}. Although there are a number of publications on the alkaloids of *T. terrestris*⁷⁻⁹, there is no report in the literature dealing with the alkaloids of *T. terrestris* growing in Turkey.

In our previous studies, diosgenin content of the different parts of *T. terrestris* growing in Turkey was determined by HPLC¹⁰; steroidal sapogenins were isolated by preparative TLC⁶ and in-vitro antibacterial activity of *T. terrestris* was screened by the microdilution method¹¹. Alkaloids of different parts of *T. terrestris* collected in the vicinity of Ankara were examined and isolated by preparative TLC in this study. Alkaloids isolated were identified on the basis of their physical and IR spectral characteristics and by comparison with authentic samples (Sigma).

Material and Methods

Plant Material

Tribulus terrestris used in this study was collected from Kazan (Ankara-Turkey) in June 1989. Herbarium specimens are preserved in "Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu (AEF No. 15215)", Ankara, Turkey.

Equipment

A titrimetric method was used to determine the total alkaloid content¹². TLC was performed on silica gel GF₂₅₄ (Merck) plates using chloroform/methanol/

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10 % ammonium hydroxyde (80/20/1.5)⁹ solvent system. Preparative TLC was carried out using the same solvent system on silica gel PF 254+366 (Merck) plates. Melting points were determined on a Buchi 510 Melting Point Apparatus and are uncorrected. IR spectra were run in KBr discs with a Perkin Elmer 1330 IR Spectrophotometer.

Extraction and Isolation of Alkaloids

Dried and powdered plant material was extracted with methanol in a Soxhlett apparatus. The extract was evaporated under vacuum and taken up in 5 % aqueous HCl and then filtered. The aqueous acidic filtrate extracted with chloroform was made basic with 25 % ammonium hydroxyde and extracted again with chloroform. The chloroform extract was dried with anhydrous Na₂SO₄ and evaporated under vacuum.

The crude alkaloidal mixture was examined by TLC. The alkaloids were isolated from the crude alkaloidal mixture by preparative TLC. The isolated alkaloids were identified by analysis of their physical and IR spectral data.

Quantitative Analysis of Total Alkaloids

A crude alkaloidal mixture was obtained from the dried and powdered plant material (20 g) with the method mentioned above. The crude alkaloidal mixture was treated with excess of 0.1 N H₂SO₄ solution and back titration was made with 0.1 N NaOH solution using methyl red as indicator. Total alkaloid content was calculated over major alkaloid (harmine). This method was carried out in the same way three times and an average result was taken.

Results and Discussion

The alkaloids of *T. terrestris* were examined by TLC and three of the detected compounds were identified as harman⁷⁻⁹, harmine^{7,8} and harmol⁹.

The total alkaloid contents of the roots, stem + leaves and fruits were found to be 0.46, 0.57 and 0.87 % respectively, by a titrimetric method. Harman, harmine and harmalol were isolated from the

roots, stem+leaves and fruits and harmaline from the roots and stem+leaves.

Harman, R_f 0.94; mp 238-9°C; IR $\bar{\nu}$ _{max} (3420, 3100-3000, 3000-2900, 1620, 1560, 1500, 1450, 1380 and 745 cm⁻¹).

Harmine, R_f 0.88; mp 263-4°C; IR $\bar{\nu}$ _{max} (3260, 3100-3000, 3000-2900, 1625, 1565, 1510, 1450, 1385, 1272, 1160, 880 and 800 cm⁻¹).

Harmaline, R_f 0.38; mp 231-2°C; IR $\bar{\nu}$ _{max} (3280, 3100-3000, 3000-2900, 1625, 1570, 1530, 1445, 1375, 1270, 1160, 900 and 805 cm⁻¹).

Harmalol, R_f 0.12; mp 211-2°C; IR $\bar{\nu}$ _{max} (3400, 3280, 3100-3000, 3000-2900, 1640, 1565, 1500 1460, 1380, 905 and 810 cm⁻¹).

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