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DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN THE SEEDS OF *HELIOTROPIUM EUROPAEUM* BYGC-MS

HELIOTROPIUM EUROPAEUM TOHUMLARINDA GC-MS İLE PİROLİZİDİN ALKALOİTLERİNİN TAYİNİ

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ABSTRACT

In the current research, alkaloid extract obtained from the seeds of Heliotropium europaeum L. collected from Köprübaşı (Diyarbakır, Turkey) was analysed by using GC-MS method. The total pyrrolizidine alkaloid and tertiary base content of the seeds of Heliotropium europaeum were found to be 0.28 % and 0.02 % respectively. Higher percentage of alkaloids were present as N-oxides (92.86 % of the alkaloids). Alkaloids found in the tertiary base fraction and total alkaloid fraction were identified as europine, heliotrine, supinine, heleurine, lasiocarpine and 7-angelylheliotrine.

Key words:: *Heliotropium europaeum, Pyrrolizidine alkaloids*

ÖZET

Bu çalışmada, Köprübaşı (Diyarbakır-Türkiye) den toplanan Heliotropium europaeum L. bitkisinin tohumlarından elde edilen alkaloit ekstresi GC-MS ile analiz edilmiştir. Tohumlarda total pirolizidin alkaloidi miktarı % 0.28, tersiyer bazların miktarı ise % 0.02 olarak bulunmuştur. Total alkaloit miktarının % 92.86 sini N-oksitleri oluşturmaktadır. Total alkaloit ve tersiyer baz fraksiyonlarındaki alkaloitler europin, heliotrin, supinin, heleurin, lasiokarpin ve 7-angelilheliotrin olarak teşhis edilmiştir.

Anahtar kelimeler: *Heliotropium europaeum, Pirolizidin alkaloitleri*

INTRODUCTION

The genus *Heliotropium* (Boraginaceae) is represented by fourteen species in the Turkish flora and two of which are endemic. *H. europaeum* is an annual herb distributed in European Turkey and North, South, East and Inner Anatolia (1). *Heliotropium* species are known to contain pyrrolizidine alkaloids which mainly spread in the genera of Boraginaceae, Asteraceae and Leguminosae families and have a wide variety of biological activities such as antitumor, antibacterial, antifungal, insecticide, antispasmodic, mydriatic, mutagenic, teratogenic and hepatotoxic activity (2).

MATERIAL AND METHODS

Plant material

Heliotropium europaeum used in this study was collected from Köprübaşı (Diyarbakır, Turkey). Herbariura specimens are preserved in "Dicle Üniversitesi Herbaryumu (DUF No. 9028), Diyarbakır and Gazi Üniversitesi Fen Fakültesi Herbaryumu-GAZİ (DUF No. 9028), Ankara, Turkey ".

Extraction of alkaloids

10 g air-dried and povvdered seeds were defatted with hexane (3 x 100 mi) and homogenised twice in 100 ml 0.5 M H_2S0_4 and left standing at room temperature for one hour each. Then, the mixture was filtered and divided into two equal portions and each was processed separately. First portion was made alkaline with 25% ammonia solution (pH 10.5) and extracted with dichloromethane (3 x 100 ml). The organic phase was dried över anhydrous Na_2S0_4 and evaporated under vacuum. The residue (10.82 mg-fraction A) was regarded as the free tertiary bases. Second portion was reduced with Zn dust, filtered, made alkaline with 25% ammonia solution (pH 10.5) and extracted with dichloromethane (3 x 100 mi). The organic phase was dried över anhydrous Na_2S0_4 and evaporated under vacuum. The residue (145.31 mg-fraction B) indicate total alkaloid (tertiary bases and N-oxides). The pyrrolizidine alkaloid content of the fraction A and B was determined by using GC-MS. External standard method was used for quantitative determination.

GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on a Hewlett-Packard model 6890 gas chromatograph combined with a Hewlett-Packard model 5972 A MS detector. The column was HP-5 5% phenylmethyl siloxane (25m x 0.20 mm ID, 0.33um film thickness). Carrier gas was helium with a 1 ml/min. flow rate. Injection temperature was 250°C. Column temperature was programmed from 120°C (3 min.) to 230°C (10 min.) at 20°C/min. to 290°C (2 min.) at 10°C/min.; splitless. Electron ionization (El) data was acquired using the following conditions: 70 eV electron energy, 190°C source temperature. Library search was carried out using Wiley GC-MS Library.

RESULTS and DISCUSSION

The total pyrrolizidine alkaloid and tertiary base content of the seeds of *Heliotropium europaeum* were found to be 0.28 % and 0.02 % respectively. Higher percentage of alkaloids were present as N-oxides (92.86 % of the alkaloids). Alkaloids found in the tertiary base fraction and total alkaloid fraction are shown in the Table.

Table. Alkaloids found in the tertiary base and total alkaloid fraction

Alkaloids	Tertiary base fraction (%)	Total alkaloid fraction (%)
Europine	19.22	14.27
Heliotrine	9.56	2.44
Supinine	46.19	9.09
Heleurine	16.79	2.65
Lasiocarpine	4.01	8.69
7-Angelylheliotrine	4.23	2.86

The presence of europine, heliotrine, lasiocarpine, heleurine and supinine in *H. europaeum* were previously reported (2-4). This is the first report of detection of 7-angelylheliotrine in *H. europaeum*. Mass spectral data of 7-angelylheliotrine, m/z: 395 (M^+, 5), 295 (7), 220 (100), 136 (57), 120 (80), 119 (70), 93 (43), 83 (34), 59 (55), 43 (18).

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REFERENCES

1. Riedel, H. *"Heliotropium"* **in Flora of Turkey and East Aegean Islands, Davis, P. H. (Ed.), Edinburgh University Press, Edinburgh, 6: 248-55 (1978).**

2. **Rizk, A-F. M.** *"The pyrrolizidine alkaloids: Plant sources and properties" in Naturally Occurring Pyrrolizidine Alkaloids, Rizk, A-F. M. (Ed.), CRC Press, Inc., Boca Raton, 1-89* **(1991).**

3. **Culvenor, C. C. J., Drummond, J. L., Price, J. R.** *"Alkaloids of* Heliotropium europaeum. */. Heliotrine and lasiocarpine". Aust. J. Chem., 7, 277-280 (1954). C. A.,* ***49,*** *8998h, 1955.*

4. **Culvenor, C. C. J.,** *"Alkaloids of* Heliotropium europaeum. *II Isolation and structures of the third major alkaloid and two minor alkaloids and isolations of the principal N-oxides". Aust. J. Chem. 7: 287-297 (1954). C.A., 49, 8999h, 1955.*