

PHYTOCHEMICAL STUDY OF *Gypsophila trichotoma* WEND. (CARYOPHYLLACEAE)

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Phytochemical studies of ethyl acetate extracts from the roots and aerial parts of *Gypsophila trichotoma* revealed the presence of stigmast-7-en-3-ol, stigmasta-7,22-dien-3-ol (spinasterol), ergost-7-en-3-ol, stigmasta-3,5-dien-7-one, β -sitosterol, 22,23-dihydrospinasterone, vitexin, orientin, homoorientin and hyperoside. The structures of the compounds were determined by GC/MS and HPLC analyses.

Keywords: *Gypsophila trichotoma*; Caryophyllaceae; flavonoids.

INTRODUCTION

The plants of genus *Gypsophila* (Caryophyllaceae) are well known by their medicinal, decorative and industrial application. The species of *Gypsophila* are studied for saponins,¹ flavonoids² and sterols.³ *Gypsophila* saponins have anticarcinogenic properties, including direct cytotoxicity, immune-modulating effects, and normalization of carcinogen induced cell proliferation.⁴ From *Gypsophila* species were also isolated Δ sterols, which showed high biological activity against hyperplasia of the prostate,⁵ an antimutagen activity,⁶ therapeutic potential to modulate the development and/or progression of diabetic nephropathy⁷ and an antitumor activity.⁸

Gypsophila trichotoma Wend. (Caryophyllaceae) is a perennial herb, located in Southeast Europe (from East Bulgaria to Southeast Russia). The plant is spread in the northeast region of Black sea coast.⁹ Previous studies of *G. trichotoma* have led to isolation of triterpene saponins.¹⁰⁻¹⁴ This report describes the identification of the steroids and flavonoids in *Gypsophila trichotoma* Wend.

EXPERIMENTAL

Plant material

The plant material (roots and aerial parts) was collected in June 2003 in the northeast region of Black sea coast, locality "Zelenka", near village Balgarevo, Bulgaria. The voucher specimen (SO 103887) was deposited in Herbarium of Sofia University, Bulgaria.

Extraction and fractionation

The dried roots of *G. trichotoma* (840 g) were exhaustively extracted with 80% MeOH and evaporated. The extracts were concentrated and the aqueous residue was extracted with Cl_2CH_2 , EtOAc and BuOH. The EtOAc extract (10 g) was purified with column chromatography over Sephadex LH-20 (MeOH) and flash chromatography over silica gel (CHCl_3 -MeOH, 9:1) to give two main purified sterol subfractions, which were submitted to GC/MS analysis.

The dried aerial parts of *G. trichotoma* (10 g) were exhaustively extracted with 80% MeOH. The MeOH extract was suspended in

water and then successively extracted with CHCl_3 and EtOAc. The EtOAc extract was subjected to column chromatography using Sephadex LH-20 eluted with MeOH to give 23 fractions, which were combined after TLC. Three main purified flavonoid subfractions were further subjected to HPLC.

Analyses of the sterols and flavonoids

GC/MS analysis

GC/MS analysis was performed on a Hewlett Packard 6890 + MS 5973 with a capillary column HP-5, 23 m \times 0.2 mm, 0.5 μm film thickness. Helium was used as a carrier gas with a temperature programme 100-315 $^\circ\text{C}$ at 5 $^\circ\text{C}$ min^{-1} and a 10-min hold at 315 $^\circ\text{C}$. GC/MS research was based on the interpretation of the mass spectral fragmentation followed by comparisons of the obtained spectra with those of authentic samples. Computer searches in a HP Mass Spectral Library NIST98 were also applied.

HPLC analysis

HPLC analysis was performed on a Spherisorb C18 ODS, column 5 μm , 250 \times 4.6 mm; mobile phases: MeOH:H₂O (60:40 v/v) (1), ACN:H₂O (70:30 v/v) (2) and ACN:H₂O (45:55 v/v) (3); flow-rate: 1 mL/min⁻¹; UV-detection at 254 nm. The chromatograms were compared with those obtained from authentic samples analysed on the same mobile phases. The reference solutions of vitexin, hyperoside, orientin, and homoorientin were prepared by dissolving an equivalent amount of each of them in a mobile phase to obtain concentration 0.00001 g/mL. The standards were obtained from Extrasynthese (Genay, France). Test solutions – the samples were added 1.0 ml from solvents methanol – water (60:40 v/v).

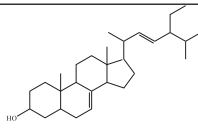
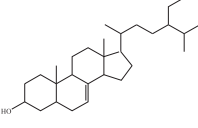
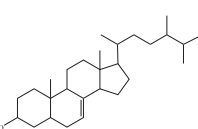
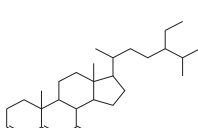
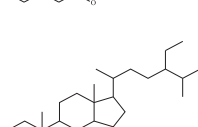
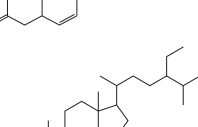
RESULTS AND DISCUSSION

The ethyl acetate extract of *G. trichotoma* roots was purified by Silica gel and Sephadex LH-20 column chromatography to give two main sterol fractions, which were further analysed by GC/MS. The investigation was based on the interpretation of the mass spectral fragmentation followed by comparisons of the obtained spectra with those of authentic samples.¹⁵ Peak identification was accomplished by a comparison of their mass spectra with those stored on the GC/MS database. Six compounds were identified as: stigmast-7-en-3-

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ol, stigmasta-7,22-dien-3-ol (spinasterol), ergost-7-en-3-ol, 24-ethylcholesta-3,5-dien-7-one, β -sitosterol and 22,23-dihydro-spinasterone (Table 1).

Table 1. Compounds identified by GC/MS

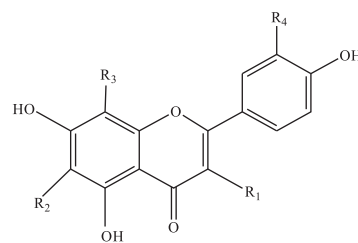
Steroids	Main mass fragments	Structure
Stigmasta-7, 22-dien-3-ol (spinasterol)	412/271	
Stigmast-7-en-3-ol	414/255	
Ergost-7-en-3-ol	400/255	
Stigmasta-3, 5-dien-7-one	410/174	
22,23-dihydro-spinasterone	412/271	
β -sitosterol	414/396	

Chromatographic separation of the ethyl acetate extract from the aerial parts over Sephadex LH-20 afforded three purified flavonoid fractions, which were analysed by HPLC. The prescribed HPLC method for analysis of flavonoids is based on literature methods for these compounds with some modifying elements – changed mobile phases and column.¹⁶⁻¹⁸ To achieve an assuredly results the efficacy of the separation were tested with reference substance in different phases. The optimised results are presented on Table 2. Mobile phase (1) is the most suitable for studying of fractions with vitexin. For fractions with vitexin and orientin the most suitable is mobile phase (2). The relative retention of compounds in mobile phase (1) is about

Table 2. Data from HPLC study of flavonoids in three mobile phases

Flavonoids	Mobile phase	t _r (min)	N (TP)*
Vitexin	1	4.23	48764
	2	2.10	10160
	3	1.76	12543
Orientin	1	4.66	19602
	2	2.86	821
Homoorientin	3	2.33	6398
Hyperoside	2	2.20	7743

*Number of theoretical plates



	R ₁	R ₂	R ₃	R ₄
Vitexin	H	H	glc	H
Orientin	H	H	glc	OH
Homoorientin	H	glc	H	OH
Hyperoside	O-gal	H	H	OH

Figure 1. Structures of the identified flavonoids

0.90 but in mobile phase (2) – 0.73. The conditions are very suitable for simultaneous determination of both compounds without preliminary separation. Using mobile phase (3) were identified vitexin and homoorientin. Four compounds were identified as hyperoside,¹⁹ vitexin,²⁰ orientin⁹⁻²⁰ and homoorientin²⁰ by comparison of the retention time of the compounds of the flavonoid fractions and standards (Figure 1).

CONCLUSIONS

Phytochemical investigation of *G. trichotoma* led to identification of six steroids and four flavonoids by GC/MS and HPLC analyses. This is the first report about the presence of these compounds in the roots and aerial parts of the plant.

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