Sterols and triterpenes in *Viburnum opulus* L. leaves

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S u m m a r y

Ursolic acid and β-sitosterol were isolated from *Viburnum opulus* L. leaves with the use of column chromatography. Their structure was determined by spectroscopic methods (IR, NMR). Other sterols and triterpenes in fractions obtained from benzine and chloroform extracts from *V. opulus* L. leaves were examined using TLC, GC, GC/MS methods. Following triterpenes there were identified: α-amyrin, β-amyrin, as well as sterols: campesterol, stigmasterol, β-sitosterol. The qualitative analysis of benzine and chloroform extract from *V. opulus* L. flowers has indicated the presence of β-sitosterol and ursolic acid.

Key words: Viburnum opulus L., *leaves, flowers, sterols, triterpenes*

INTRODUCTION

Viburnum opulus L. is a medium-sized to large-sized shrub common in Poland. It is also cultivated as an ornamental plant [6, 7].

The dried bark of stem is the part of the plant used in medicine, especially as a muscle relaxant as well as an astringent and antiinflammatory agent [1, 7-9]. It is helpful in muscular aches and conditions associated with smooth muscle tension and high blood pressure as well as to relieve menstrual cramps and muscle spasms. Bark has been traditionally used in cases of threatened miscarriage and in treatment of hemoroids [1, 7, 8]. Similar abilities are also shown by leaves, fruits and flowers of *V. opulus* L. [7, 9].

Known components of bark and fruits of *V. opulus* L. are catechine tannins [1, 7], coumarins (scopoletin, esculetin) [2, 7, 10], flavonoids (astragalin, kaempferol, quercetin, amentoflavon), sterols, triterpenes [2, 7]. The chemical composition of leaves and flowers has been significantly less investigated so far.

MATERIAL AND METHODS

The leaves and flowers of *V. opulus* L. collected in the Botanical Garden in Łódź in May and June 2003 and dried in natural conditions. Their samples are deposited in the herbarium of the Department of Pharmacognosy of the Medical University in Łódź

Dried powdered plant material (leaves – 300 g, flowers – 405 g) was successively extracted with benzine (EBeL-20g, EBeF-21g) and chloroform $CHCl_3$ (EChL- 18g, EChF-29g) in a Soxhlet apparatus.

Column chromatography (CC) was carried out using silica-gel (MN-Kieselgel 60 grain size 0.05–0.2 nm, 70–270 mesh ASTM) filled columns and solvent system of increasing polarity. TLC analysis was carried out using silica-gel precoated plates (with 0.25 mm layer thickness) – Kieselgel G60 (Merck). Standard solution: β-sitosterol, α-amyrin, β-amyrin, ursolic acid (Fluka, Switzerland), oleanic acid (Roth, Germany), 10 mg each, were diluted with 10 cm^3 of MeOH. After application of extract and standard solution (approx. 50 μl each), plates were developed to a distance of 18 cm in all-glass chambers. The chambers were conditioned for 30 min. with mobile phase vapor. After development the mobile phase was evaporated to dryness. Chromatograms were analyzed in daylight and in $UV_{\lambda=366,254\ nm}$ after spraying with reagent R1 and heating 110°C for 10 min. The reagent: R1 - Liebermann-Burchard reagent (20ml MeOH + 2ml conc. H_2SO_4 + 2ml acetic anhydride)

The solvent systems: CC: S1 – Et_2O:C_6H_6 (step gradient), S2 – C_6H_6:$CHCl_3$ (step gradient), S3 – $CHCl_3$: AcOEt (step gradient); TLC: S4 – C_6H_6:hexane 20:30, S5 – C_6H_6:$CHCl_3$:MeOH 20:30:4

Gas chromatography was carried out using:

GAS CHROMATOGRAPHY (GC): Carlo Erba chromatograph, model MEGA HRGC 5300 equipped with a flame-ionization detector (FID), SSL injector and with fused stationary phase OV-1 capillary column (25 m x 0.32 mm) i.d. film thickness 0.1 μm.

The conditions of GC analyses:

- temperature programme from 200°C to 300°C at 6°C/min.
- flow rate of the carried gas (N_2) was 1.5 ml/min.
- temperature of injector 320°C
- temperature of detector 310°C

GAS CHROMATOGRAPHY WITH MASS SPECTROMETER (GC/MS): Fisons chromatograph GC 8000 combined with MD 800 mass spectrometer with the same capillary column as mentioned above.

The conditions of GC/MS analyses:
– temperature program from 200°C to 300°C at 6°C/min.
– flow rate of the carried gas (helium) was 1.5 ml/min.
– electron impact energy 70eV.
– temperature of injector 320°C
– temperature of detector 310°C
Spectra were recorded with the following instruments:
– NMR Bruker – DRX-500, ^{1}H – (500,13 MHz), ^{13}C – (125,76 MHz),
– IR spectrometer ATI Mattson Infinity series FTIR

Separation and identification of sterols and triterpenes

Benzine extract EBeL (20 g) was separated on silica-gel (600 g) filled columns (Ø 5.5 cm x 100 cm), using solvent systems of increasing polarity S1 – Et_2O:C_6H_6, S2 – C_6H_6:$CHCl_3$, S3 – $CHCl_3$: AcOEt. Separation on those columns was controlled with TLC using solvent systems S4 and S5. From residue of fractions eluted with benzene: chloroform 2:8 (MEBeL), after crystallization from EtOH the compound **I** was obtained. In mother liquor of MEBeL mixture of sterols was examined.

Chloroform extract EChL (18 g) was separated on silica-gel (400 g) filled columns (Ø 4.5 cm x 85 cm), using solvent systems of increasing polarity S1 – Et_2O:C_6H_6, S2 – C_6H_6:$CHCl_3$, S3 – $CHCl_3$: AcOEt. Separation on those columns was controlled with TLC using solvent systems S4 and S5. From initial fractions eluted with petroleum ether: benzene 1.5:8.5, fractions containing a mixture of triterpenes MEChL were obtained. From residue of further fractions eluted with chloroform:ethyl acetate 5.5:4.5, after crystallization from EtOH, compound **II** was obtained.

The structures of compound **I** and **II** were identified by spectroscopic methods (IR, NMR). Other sterols and triterpenes in fractions obtained from benzine and chloroform extracts from leaves of *V. opulus* L. were examined using TLC, GC and CG/MS methods.

The sterols were identified by comparison of the retention times and mass spectra of the analyzed compounds with authentic standards and literature data from NIST MS Library. The results of GC, GC/MS analysis of fractions MEBeL and MEChL are presented in table 1, the example chromatograms in figure 1 and figure 2.

Compound **I –** β-sitosterol, colourless needles, m.p. 136–140˚C

Compound **II –** ursolic acid, beige needles, m.p. 264–268˚C

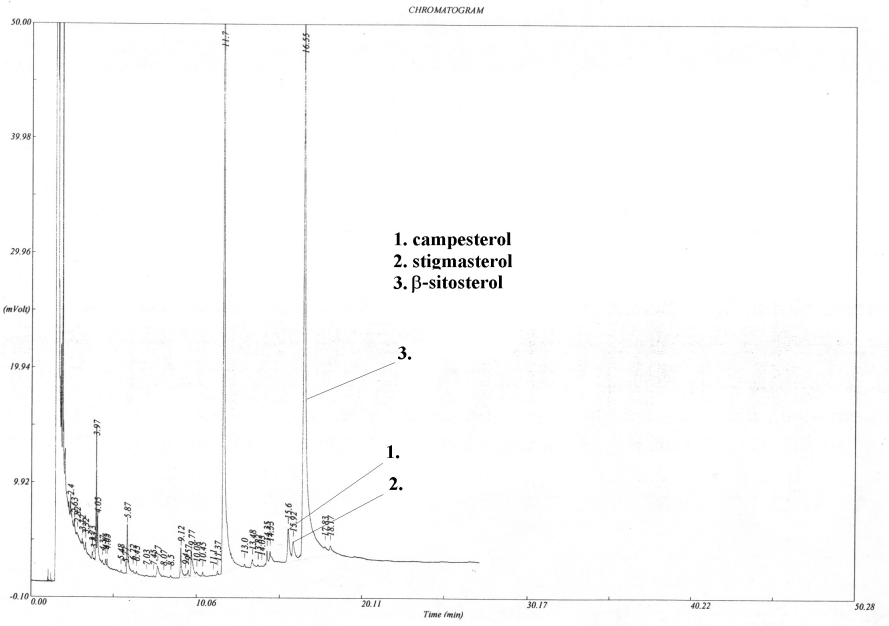

Figure 1. GC chromatogram of MEBeL fraction

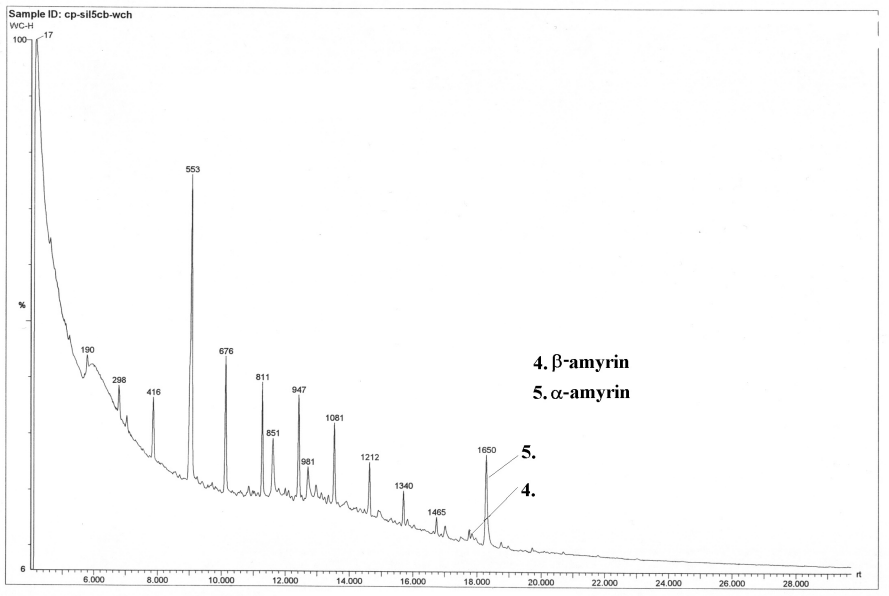

Figure 2. GC/MS chromatogram of MEChL fraction

Table 1.

Sterols and triterpenes from *V. opulus* L. identified in MEBeL and MEChL fractions by GC and GC/MS methods

peak	t_R (min.)	M^+ (m/z)	identified compounds	
			MEBeL	MEChL
1.	15.60	401	campesterol	----------
2.	15.92	413	stigmasterol	----------
3.	16.55	415	β-sitosterol	----------
4.	17.32	427	----------	β-amyrin
5.	17.63	427	----------	α-amyrin

Spectra ^{1}H NMR, ^{13}C NMR, IR of compounds **I** and **II** are conformable with published values of NMR, IR data [3-5].

The chromatographic analysis (TLC) of sterols and triterpenes from flowers of V. opulus L.

The presence of sterols and triterpenes in EBeF and EChF from flowers of *V. opulus* L. was recognized by comparison with standards: stigmasterol, β-sitosterol, α-amyrin, β-amyrin, ursolic acid and oleanic acid. After distilling the solvent the residues from EBeF and EChF were diluted with 10 cm^3 of MeOH. Sterols and triterpenes were examined chromatographically using TLC with solvent S5. Chromatograms were visualized after spraying with R1 – Liebermann-Burchard reagent. The qualitative analysis has indicated the presence of β-sitosterol and ursolic acid.

RESULTS AND DISCUSSION

The aim of the research was the thorough examination of the sterolic and triterpene compounds in leaves and the qualitative analysis of sterols and triterpenes in *V. opulus* L. flowers.

Using column chromatography on silica-gel from benzine the EBeL extract from leaves of *V. opulus* L. chromatographically homogenous β-sitosterol (compound **I**) and from chloroform extract EChL ursolic acid (compound **II**) were isolated. Their structure was determined by spectroscopic methods (IR, NMR). Compounds **I** and **II** were identified by comparison of their NMR data with published values. Additionally to that both benzine and chloroform extracts gave fractions containing mixture of sterols and triterpenes and they were examined by GC, CG/MS methods. Following triterpenes were identified: α-amyrin, β-amyrin, along with sterols: campesterol, stigmasterol and β-sitosterol. The compositions of triterpene and sterolic fractions from flowers of *V. opulus* L. were investigated chromatographically using TLC with the S5 solvent. The qualitative analysis has indicated the presence of β-sitosterol and ursolic acid.

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STEROLE I TRITERPENY W LIŚCIACH *VIBURNUM OPULUS* L.

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S t r e s z c z e n i e

W wyniku rozdziałów prowadzonych metodą chromatografii kolumnowej na żelu krzemionkowym z wyciągu benzynowego EBeL z liści kaliny koralowej wyizolowano chromatograficznie jednorodny związek **I** – β-sitosterol, natomiast z wyciągu chloroformowego EChL chromatograficznie jednorodny związek **II** – kwas ursolowy. Ponadto w wyniku rozdziałów wyciągów benzynowego i chloroformowego otrzymano frakcje zawierające mieszaninę steroli i triterpenów, które dalej badano metodą chromatografii gazowej GC i chromatografii gazowej sprzężonej ze spektrometrią masową GC/MS. We frakcjach z EBeL, oprócz

β-sitosterolu, stwierdzono obecność kampesterolu i stigmasterolu, we frakcjach z EChL stwierdzono obecność związków z grupy triterpenów: α-amyryny i β-amyryny. Prowadzona metodą TLC analiza jakościowa wyciągu benzynowego EBeF i chloroformowego EChF z kwiatów kaliny koralowej wykazała obecność β-sitosterolu i kwasu ursolowego.

Słowa kluczowe: Viburnum opulus L., liście, kwiaty, sterole, triterpeny