ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 8:3 (2014) 228-233

records of natural products

Saponins from *Cephalaria aristata* C. Koch

Derya Gülcemal[*1], Milena Masullo[2], Serdar Gökhan Şenol[3], Özgen Alankuş-Çalışkan[1], and Sonia Piacente[*2]

[1]*Ege University, Faculty of Science, Department of Chemistry, Bornova, 35100, İzmir, Türkiye*

[2]*Salerno University, Department of Pharmacia, 84084 Fisciano (Salerno), Italy*

[3]*Department of Biology, Faculty of Science, Ege University, Bornova, 35100 İzmir, Türkiye*

(Received June 11, 2013; Revised September 15, 2013; Accepted October 24, 2013)

Abstract: One new oleanane-type saponin, 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosylhederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**1**) was isolated from the MeOH extract of whole plant parts of *Cephalaria aristata* C. Koch along with three known oleanane-type saponins, 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-(β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl) ester (**2**), 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin (**3**) and 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosylhederagenin (**4**). Also three triterpenoids and a steroid glucoside oleanolic acid (**5**), β-amyrin (**6**), 20-β-hydroxyursolic acid (**7**) and 29-hydroxystigmast-5-en-3-*O*-β-D-glucopyranoside (**8**). Their structures were established by the extensive use of 1D- and 2D-NMR experiments along with ESIMS and HRMS analysis.

Keywords: *Cephalaria aristata*; Dipsacaceae; Oleanane-type saponins.

1. Introduction

The genus *Cephalaria* (Dipsacaceae) consists of 94 species distributed mainly in the Mediterranean region and the Middle East. In Turkey, there are 40 *Cephalaria* taxa and 24 of them are endemic [1-3]. *Cephalaria aristata* is an annual herb which mainly grows at inner and east Anatolia, also Armenia [2]. Several *Cephalaria* species have been used as folk medicine for their hypothermal, alleviative, relaxant and anti-infective activities [4,5]. Flavonoids [6,7], iridoids [8] and triterpene saponins with antimicrobial [9-11], antifungal [9], molluscicidal [10] and cytotoxic activities [10,12] have been previously reported from this genus. As a part of our ongoing research of new compounds from Turkish Dipsacaceae species [A-D] [13-16] we carried out a study on *Cephalaria aristata* C. Koch.
This paper reports the isolation of one new oleanane-type triterpene glycoside (**1**) from the methanol extract of the whole plant of *C. aristata* along with three known oleanane-type glycosides (**2-4**), three aglycones (**5-7**) and one sterol glucoside (**8**). Their structures were elucidated by extensive

[*] Corresponding author: E-mail: derya.gulcemal@ege.edu.tr; Phone +902323882369 fax: +902323888264; e-mail: piacente@unisa.it; Phone: ++39089969763fax: ++39089969602 .

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 05/01/2014 EISSN:1307-6167

spectroscopic methods including 1D- (^{1}H, ^{13}C and TOCSY) and 2D-NMR (DQF-COSY, HSQC, HMBC and ROESY) experiments as well as ESIMS and HRMS analysis.

2. Materials and Methods

2.1. General procedures

Optical rotations were measured on a JASCO DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpinGmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbeat 300 K. All 2D-NMR spectra were acquired in CD_3OD (99.95%, SigmaAldrich) and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. The NMR data were processed using UXNMR software. Exact masses were measured by a Voyager DE mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDITOF) mass spectrometry. A mixture of analyte solution and a-cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18-39) at 2465.1989 Da and angiotensin III at 931.5154 Da as internal standard. ESIMS analyses were performed using a ThermoFinnigan LCQ Deca XP Max iontrap mass spectrometer equipped with Xcalibur software.

2.2. Plant Material

Cephalaria aristata C. Koch was collected from Darende-Malatya, Turkey in June 2010 and identified by one of the co-author, Serdar G. Şenol (Deparment of Biology, Faculty of Sciences, Ege University, İzmir, Turkey). A voucher specimen (EGE 40857) is deposited in the Ege University Botanical Garden & Herbarium Research and The Application Center, İzmir, Turkey.

2.3. Extraction and Isolation

Air-dried and grinded plant material (900 g) were extracted with MeOH (4x5 L) at room temperature. After filtration, the solvent was removed by rotary evaporation yielding (60 g) of MeOH extract. The MeOH extract was dissolved in H_2O (350 ml), and successively partitioned with n-hexane (3x150 mL), CH_2Cl_2 (4x150 mL), and n-BuOH saturated with H_2O (4x150 mL). The n-BuOH phase (10 g) was chromatographed over Sephadex column chromatography to give ten main fractions (A-J). Fraction A (2.5 g) was subjected to vacuum liquid chromatography (VLC) using reversed-phase material (RP-18) employing H_2O (600 mL), H_2O-MeOH (8:2, 1000 mL; 6:4, 1000 mL; 4:6, 1500 mL; 3:7, 1000 mL; 2:8, 1200 mL) and MeOH (800 mL) to give 6 main fractions. Fraction 2 (50 mg) was subjected to RP-HPLC column chromatography with the solvent system MeOH:H_2O (50:50, 250 ml) to yield compound **1** (3 mg). Fraction 3 (50 mg) was submitted to RP-HPLC column chromatography with the solvent system MeOH:H_2O (60:40, 250 ml) to yield compounds **2** (3 mg) and **3** (2 mg). Fraction 4 (60 mg) was subjected to RP-HPLC column chromatography with the solvent system MeOH:H_2O (70:30, 250 ml) to yield compound **4** (3 mg). Fraction 5 (50 mg) was subjected to RP-HPLC column chromatography with the solvent system MeOH:H_2O (90:10, 200 ml) to yield compounds **5** (3 mg), **6** (4 mg) and **7** (3 mg). Fraction 6 (40 mg) was submitted to RP-HPLC column chromatography with the solvent system MeOH:H_2O (95:15, 250 ml) to yield compound **8** (2 mg).

2.4. Acid hydrolysis

The crude saponin mixture (300 mg) was heated at 60 °C with 1:1 0.5 N HCl-dioxane (100 ml) for 2 h, and the mixture was then evaporated in vacuo. The residue was partitioned with CH_2Cl_2-H_2O, and the H_2O layer was neutralized with Amberlite MB-3. The H_2O layer was then concentrated and passed through a silica gel column, using $CHCl_3$-MeOH-H_2O (7:1:1.2, lower layer) as eluting solvent to afford glucose, xylose, arabinose and rhamnose. The D configuration of xylose and glucose and the L configuration of arabinose and rhamnose were established as by comparison of their optical rotation

values with those reported in the literature [17] (Eskander et al., 2006). The optical rotations were determined after dissolving the sugars in H_2O and allowing them to stand for 24 h; D-xylose $[\alpha]^{22}_D$ +10 (*c* 0.1), L-arabinose $[\alpha]^{22}_D$ +80.5 (*c* 0.1), D-glucose $[\alpha]^{22}_D$ +51.0 (*c* 0.1), L-rhamnose $[\alpha]^{22}_D$ +13.8 (*c* 0.1).

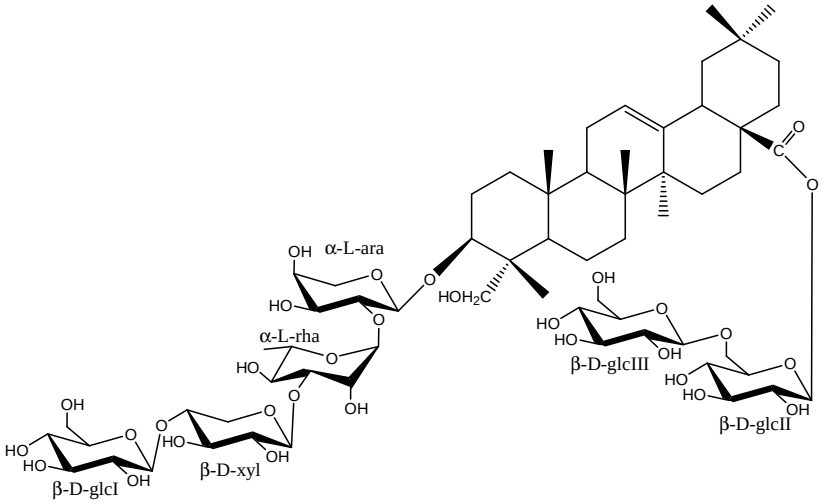

Figure 1.Structure of compound **1**

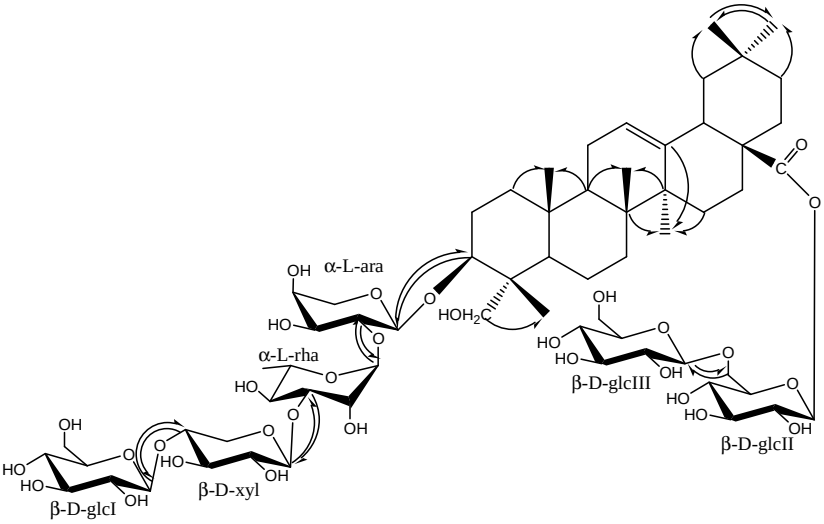

Figure 2.Key HMBC of compound **1**

3. Results and Discussion

3.1. Structure elucidation

The HRMALDITOF mass spectrum of **1** (*m/z* 1391.6465 $[M+Na]^+$, calcd for $C_{64}H_{104}O_{31}Na$, 1391.6459) supported a molecular Formula of $C_{64}H_{104}O_{31}$. The ESIMS spectrum showed a major ion peak at *m/z* 1391 which was assigned to $[M+Na]^+$. The MS/MS of this ion showed a peak at *m/z* 1229 $[M+Na-162]^+$, corresponding to the loss of a hexose unit. In the MS^3 spectrum peaks at *m/z* 1067

$[M+Na-162-162]^+$, corresponding to the loss of a hexose unit, 1023 $[M+Na-162-162-44]^+$, due to consecutive losses of carbon dioxide, 861 $[M+Na-162-162-44-162]^+$, corresponding to the loss of a hexose unit, 729 $[M+Na-162-162-44-162-132]^+$, corresponding to the loss of a pentose unit, 583 $[M+Na-162-162-44-162-132-146]^+$, corresponding to the loss of a deoxy-hexose unit, 451 $[M+Na-162-162-44-162-132-146-132]^+$, corresponding to the loss of a pentose unit were observed.

The ^{1}H NMR spectrum of **1** displayed signals for six tertiary methyl groups at δ 0.74, 0.83, 0.94, 0.97, 1.00 and 1.21, for an olefinic proton at δ 5.27 (t, *J*=3.5 Hz), one oxygen-bearing methine proton at δ 3.64 (dd, *J*=11.5, 4.2 Hz, H-3) and one primary alcoholic function at δ 3.36, 3.61 (H-23) (Table 1). These signals along with the carbon resonances in the ^{13}C NMR spectrum for the methyl groups at δ 13.8, 17.7, 33.5, 23.8, 16.6, 26.3 and the two olefinic carbons at δ 123.5, 146.3 suggested that compound **1** possessed hederagenin as aglycon [18]. The downfield shifts of C-3 (δ 82.2) and C-28 (177.7) of the aglycon suggested that compound **1** was a bidesmosidic glycoside. The ^{13}C NMR spectrum showed 64 carbon signals, of which 30 were assigned to the aglycon moiety and 34 to a sugar portion made up of six sugar units. The ^{1}H NMR spectrum displayed in the sugar region signals corresponding to six anomeric protons at δ 4.37 (d, *J*=7.5 Hz), 4.37 (d, *J*=7.5 Hz), 4.54 (d, *J*=7.5 Hz), 4.55 (d, *J*=3.7Hz), 5.26 (d, *J*=1.2 Hz) and 5.38 (d, *J*=7.5 Hz) which were unambiguously correlated by HSQC experiment to the corresponding carbon resonances at δ 103.2, 104.5, 105.8, 104.5, 100.9 and 95.3, respectively. The chemical shifts of all the individual protons of the six sugar units were ascertained from a combination of 1D-TOCSY and DQF-COSY spectral analysis, and the ^{13}C chemical shifts of their relative attached carbons were assigned unambiguously from the HSQC spectrum (Table 1). These data showed the presence of three β-glucopyranosyl units (δ 4.37, 2H and 5.38), one α-rhamnopyranosyl unit (δ 5.26), one β-xylopyranosyl unit (δ 4.54) and one α-arabinopyranosyl unit (δ 4.55). An unambiguous determination of the sequence and linkage sites was obtained from the HMBC spectrum, which showed key correlation peaks between the proton signal of δ H-1_{ara} (δ 4.55) and the carbon resonance of C-3 (δ 82.2), H-1_{rha} (δ 5.26) and C-2_{ara} (δ 75.9), H-1_{xyl} (δ 4.54) and C-3_{rha} (δ 82.2), H-1_{glcI} (δ 4.37) and C-4_{xyl} (δ 78.1), H-1_{glcII} (δ 5.38) and C-28 (δ 177.7) and H-1_{glcIII} (δ 4.37) and the carbon resonance of C-6_{glcII} (δ 69.2). The absolute configurations of the sugar units was determined by acid hydrolysis of the crude saponin mixture as D-glucose, D-xylose, L-rhamnose and L-arabinose and were assigned on the basis of their optical rotation values [17].

On the basis of all these evidence, the structure of the new compound **1** was established as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosylhederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Additionally, three known oleanane-type triterpene glycosides; 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosylhederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**2**) [19], 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosylhederagenin (**3**) [20] and 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin (**4**) [21], three aglycones; oleanolic acid (**5**), β-amyrin (**6**) [22] and 20β-hydroxyursolic acid (**7**) [23] and one sterol glucoside, 29-hydroxystigmast-5-en-3-*O*-β-D-glucopyranoside (**8**) [24], were isolated.

Table 1. ^{13}C and ^{1}H NMR data (*J* in Hz) of the aglycon and sugar moieties of compound **1** (600Mz, δ ppm, in CD_3OD).

Aglycone			Sugar		
Position	δ_C	δ_H (*J* in Hz)	**Position**	δ_C	δ_H (*J* in Hz)
1	39.7	1.65, 0.99, m			α-L-Ara I (at C-3)
2	26.5	1.90, 1.79 m	1	104.5	4.55, d (3.7)
3	82.2	3.64, dd (11.5, 4.2)	2	75.9	3.73, dd (8.5, 3.7)
4	43.7	-	3	73.9	3.71, dd (8.5, 3.0)
5	48.0	1.30, m	4	69.6	3.78, m
6	18.7	1.51, 1.40	5	65.4	3.87 dd (11.9, 2.0)
					3.54, dd (11.9, 3.0)
7	33.1	1.64,1.31, m			α-L-Rha (at C-2_{ara})
8	40.6	-	1	100.9	5.26, d (1.2)
9	48.8	1.66, m	2	71.4	4.12, dd (1.2, 3.2)
10	37.3	-	3	82.2	3.89, dd (3.2, 9.7)
11	24.4	1.93, (2H) m	4	72.3	3.59, t (9.7)
12	123.5	5.27, t (3.5)	5	69.7	3.95, m
13	146.3	-	6	17.8	1.26, d (6.5)
14	42.8	-			β-D-Xyl I(at C-3_{rha})
15	28.7	1.86, 1.05, m	1	105.8	4.54, d (7.5)
16	23.8	1.95, 1.63, m	2	74.7	3.38, dd (7.5, 9.2)
17	47.5	-	3	75.6	3.52, t (9.2)
18	42.3	2.89, m	4	78.1	3.72, m
19	47.1	1.74, 1.18, m	5	64.3	4.07, dd (5.2, 11.7) 3.34, t (11.7)
20	31.3	-			β-D-GlcI (at C-4_{xyl})
21	34.7	1.43, 1.25, m	1	103.2	4.37, d (7.5)
22	33.2	1.766, 1.64, m	2	74.5	3.23, dd (7.5, 9.0)
23	64.5	3.61, d (11.5)	3	77.7	3.38, t (9.0)
		3.36, d (11.5)			
24	13.8	0.74, s	4	71.3	3.31, t (9.0)
25	16.6	1.00, s	5	78.0	3.35, ddd (3.5, 4.5, 9.0)
26	17.7	0.83, s	6	62.4	3.88, dd(3.5, 12)
					3.68, dd (4.5, 12
27	26.3	1.21, s			β-D-GlcII (at C-28)
28	177.7	-	1	95.3	5.38, d (7.5)
29	33.5	0.94, s	2	73.8	3.36, dd (7.5, 9.0)
30	23.8	0.97, s	3	77.9	3.44, t (9.0)
			4	70.5	3.46, t (9.0)
			5	77.6	3.53, ddd (3.5, 4.5, 9.0)
			6	69.2	4.15, dd(3.5, 12)
					3.81, dd (4.5, 12
					β-D-GlcIII (at C-6_{glcII})
			1	104.5	4.37, d (7.5)
			2	74.8	3.24, dd (7.5, 9.0)
			3	77.8	3.33, t (9.0)
			4	71.3	3.31, t (9.0)
			5	77.8	3.26, ddd (3.5, 4.5, 9.0)
			6	62.4	3.88, dd (3.5, 12)
					3.68, dd (4.5, 12)

References

[1] V. A. Matthews (1972). *Cephalaria* Schrader ex Roemer & Schultes, In: Flora of Turkey and the East Aegean Islands, ed: Davis, P.H., Edinburgh University Press, Edinburgh, vol. 4, pp. 585-597.

[2] P. H. Davis, R. R. Mill, and K. Tan (1988). *Cephalaria* Schrader ex Roemer & Schultes, In: Flora of Turkey and the East Aegean Islands, eds: P. H. Davis, R. R. Mill and K. Tan, Edinburgh University Press, Edinburgh, vol. 10, p. 156.

[3] H. Duman (2000). *Cephalaria* Schrader ex Roemer & Schultes, In: Flora of Turkey and the East Aegean Islands, eds: A. Güner, N. Ozhatay, T. Ekim and K. H. C. Başer, Edinburgh University Press, Edinburgh, vol 11, pp. 147-149.

[4] L. D. Zvidadze, G. E. Dekanosidze, O. D. Dzhikiya, E. P. Kemertelidze and A. S. Shashkov (1981). Triterpene glycosides of *Cephalaria gigantea*. II. Structure of giganteasides D and G, *Bioorg. Khim*., **7**, 736-740.

[5] D. Godevac, N. Menkovic, L. Vujisic, V. Tesevic, V. Vajs and S. Milosavljevic (2010). A new triterpenoid saponin from the aerial parts of *Cephalaria ambrosioides*, *Nat. Prod. Res*., **24**, 1307–1312.

[6] I. S. Movsumov, E. A. Garaev and M. I. Isaev (2006). Flavonoids from *Cephalaria gigantea* flowers, *Chem. Nat. Compd*., **42**, 677-680.

[7] I. S. Movsumov, E. A. Garaev and M. I. Isaev (2009). Flavonoids from *Cephalaria grossheimii*, *Chem. Nat. Compd*., **45**, 422-423.

[8] D. Godevac, B. Mandic, V. Vajs, V. Tesevic, N. Menkovic, P. Janackovic and S. Milosavljevic (2006). Triterpenoid saponins and iridoid glycosides from the aerial parts of *Cephalaria pastricensis*, *Biochem. Syst. and Ecol.*, **34**, 890-893.

[9] S. Kirmizigul, H. Anil, F. Ucar and K. Akdemir (1996). Antimicrobial and antifungal activities of three new triterpenoid glycosides, *Phytother. Res*., **10**, 274-276.

[10] S. Pasi, N. Aligiannis, H. Pratsinis, A. L. Skaltsounis and I. B., Chinou (2009). Biologically active triterpenoids from *Cephalaria ambrosioides*, *Planta Med*., **75**, 163-167.

[11] N. B. Sarıkahya and S. Kırmızıgül (2012). Antimicrobially active hederagenin glycosides from *Cephalaria elmaliensis*. *Planta Med*., **78 (8)**: 828-833.

[12] N. Tabatadze, R. Elias, R. Faure, P. Gerkens, M. C. Pauw-Gillet, E Kemertelidze, A. Chea and E. Ollivier (2007). Cytotoxic triterpenoid saponins from the roots of *Cephalaria gigantea*, *Chem. Pharm. Bull*., **55**, 102-105.

[13] O. Alankus-Caliskan and H. Anil (1995). A bidesmosidic triterpene saponin from *Cephalaria transsylvanica*, *Phytochemistry*, **38**, 1493-1495.

[14] O. Alankus-Caliskan, S. Emirdag, E. Bedir, S. Avunduk and H. Anil (2004). Triterpene saponins from *Knautia integrifolia* var. *bidens*, *Z. Naturforsch B*., **59**, 821-824.

[15] E. Polat, O. Alankus-Caliskan, T. Karayildirim and E. Bedir (2010). Iridoids from *Scabiosa atropurpurea* L. subsp *maritima* Arc. (L.), *Biochem. Syst. and Ecol.*, **38**, 253-255.

[16] D. Gulcemal, M. Masullo, O. Alankus-Caliskan, T. Karayildirim, S. G. Senol, S. Piacente and E. Bedir (2010). Monoterpenoid glucoindole alkaloids and iridoids from *Pterocephalus pinardii*, *Magn. Reson. Chem.*, **48**, 239-243.

[17] J. Eskander, C. Lavaud, I. Pouny, H. S. M. Soliman, S. M. Abdel-Khalik and I. I. Mahmoud (2006). Saponins from the seeds of *Mimusops laurifolia*, *Phytochemistry*, **67**, 1793–1799.

[18] K. Tori, S. Sera, A. Shimaoka and Y. Tomita (1974). Carbon-13 NMR spectra of olean-12-enes. Full signal assignments including quaternary carbon signals assigned by use of indirect ^{13}C, ^{1}H spin couplings, *Tetrahedron Lett*., **15**, 4227–4230.

[19] X. C. Li, C. R. Yang, Y. Q. Liu, R. Kasai, K. Ohtani, K. Yamashaki, K. Miyahara and K. Shingu (1995). Triterpenoid glycosides from *Anemoclema glaucifolium*, *Phytochemistry*, **39**, 1175–1179.

[20] D. A. Panov, V. I. Grishkovets, V. V. Kachala and A. S. Shashkov (2006). Triterpene glycosides from *Kalopanax septemlobum*. VI. Glycosides from leaves of *Kalopanax septemlobum* var. *typicum* introduced to Crimea, *Chem. Nat. Compd.*, **42**, 49-54.

[21] J. Kong, X. G. Li, B. Y. Wei and C. R. Yang (1993). Triterpenoid glycosides from *Decaisnea fargesii*, Phytochemistry, **33**, 427–30.

[22] S. B. Mahato and A. P. Kundu (1994). ^{13}C NMR spectra of pentacyclic triterpenoidssa compilation and some salient features, *Phytochemistry*, **37**, 1517-1575.

[23] O. V. Makarova and M. I. Isaev (1997). Isoprenoids of *Sambucus nigra*, *Chem. Nat. Compd.*, **33**, 702-703.

[24] A. Rashid, S. Firdous, V. U. Ahmad, K .M. Khan, S. B. Usmani and M. Ahmed, (1999). A new glycoside from *Polianthes tuberosa* Linn., *Pakistan J. Sci. Ind. R*., **42**, 26.