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Cytotoxic and immunomodulator potential of hederagenin saponins from *Cephalaria tchihatchewii*

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ABSTRACT

Two newly described oleanane-type saponins (**1–2**), named tchihatchewosides A–B, along with thirteen known compounds, were isolated from the aerial parts of *Cephalaria tchihatchewii* using several type of chromatography processes. The structures of all compounds (**1-15**) were determined by spectroscopic (1D- and 2D-NMR, HR-ESI/MS) and chemical methods. The cytotoxic activities on CCD 34Lu, A549, CRL5807, CRL 5826, HTB-177 and HeLa cell lines of newly described compounds **1–2** and **1a-2a** were evaluated by MTT method using doxorubicin as positive control. Immunomodulatory activity was performed with PMA plus ionomycin in stimulated whole blood cells treated with saponins. The supernatant was analyzed for IL-4, IFN-γ and IL1β cytokines by ELISA. DMSO was considered as the negative control. The results showed that the only compound **1a** was exhibited moderate cytotoxicity against CRL 5826 and CCD 34Lu cells. The compounds **1–2** and prosapogenins **1a-2a** stimulated IL1β cytokine release, indicating that they might potentially stimulate the innate immune response.

1. Introduction

According to the World Health Organization (WHO), about 80 % of world's population use traditional medicine for their primary health-care needs. Herbal medicine as an alternative care is reported to be still highly in use (Ajose, 2007; Rosenbloom et al., 2011). Plant species like *Cephalaria* (Caprifoliaceae) were reported to be used as folk medicines for their different biological activities (Gerkens et al., 2007). *Cephalaria* was originated from the Greek word "kephale" (head), which was known to be characterized by an arrangement of its flowers in the form of head. The main diversity of *Cephalaria* species was particularly in 2 different regions; South Africa and the Holarctic Kingdom. According to different reports, 94 species of *Cephalaria* plants have been discovered in the world. The total number of taxa of *Cephalaria* reported from Turkey is 40, from which 24 are endemic (Davis et al., 1988). The antimicrobial, antifungal, immunomodulatory, hemolytic and cytotoxic activities of *Cephalaria* species were reported along with its traditional medical usages (Kirmizigul et al., 1996; Sarikahya et al., 2018; Sarikahya and Kirmizigul, 2012, 2010). It is the case of saponins, flavonoids, iridoids and tannins which are chemically reacting and stimulating different type of biological activities. Saponins are the main compounds in the content of *Cephalaria* species. They originated from *Saponaria* plants, traditionally used as soap, are naturally known as glycosides with foaming characteristic. They were considered as a part of plant defense systems against insects and pests (Morrissey and Osbourn, 1999). In recent researches, saponins were reported to be active compounds in herbal medicines with a high contribution in food and health. Comparing to other secondary metabolites, they have several biological activities due to their structural diversity (Lacaille--Dubois and Wagner, 1996; Riguera, 1997; Sparg et al., 2004; Yoshiki et al., 1998). Various researches showed that saponins play an important role in regressing the cancer risks in the body, by mostly decreasing the cholesterol level in the blood (Desai et al., 2009). Therefore; the first detailed isolation and structural elucidation study on *Cephalaria tchihatchewi* Hub.-Mor. & Matthews, (Davis et al., 1988) which is an endemic plant and contains high amount of saponins, was carried out. The study resulted in thirteen known compounds along with two newly described triterpene saponins, named tchihatchewosides A–B (**1–2**) [Fig. 1]. The structures of the newly described triterpene glycosides were determined as; 3-*O*-*α*-L-arabinopyranosyl hederagenin

Abbreviations: A549, human alveolar adenocarcinoma; BuOH, butanol; CC, column chromatography; CCD 34Lu, normal lung fibroblast cells; CRL5807, bronchioalveolar carcinoma, non-small cell lung cancer; CRL 5826, squamous cell carcinoma, mesothelioma; HeLa, human cervix adenocarcinoma; HTB-177, carcinoma, large cell lung cancer; MeOH, methanol; MTT, [3-(4,5-dimethyl-2-thiazolyl-2,5-diphenyl-2H-tetrazolium bromide)].

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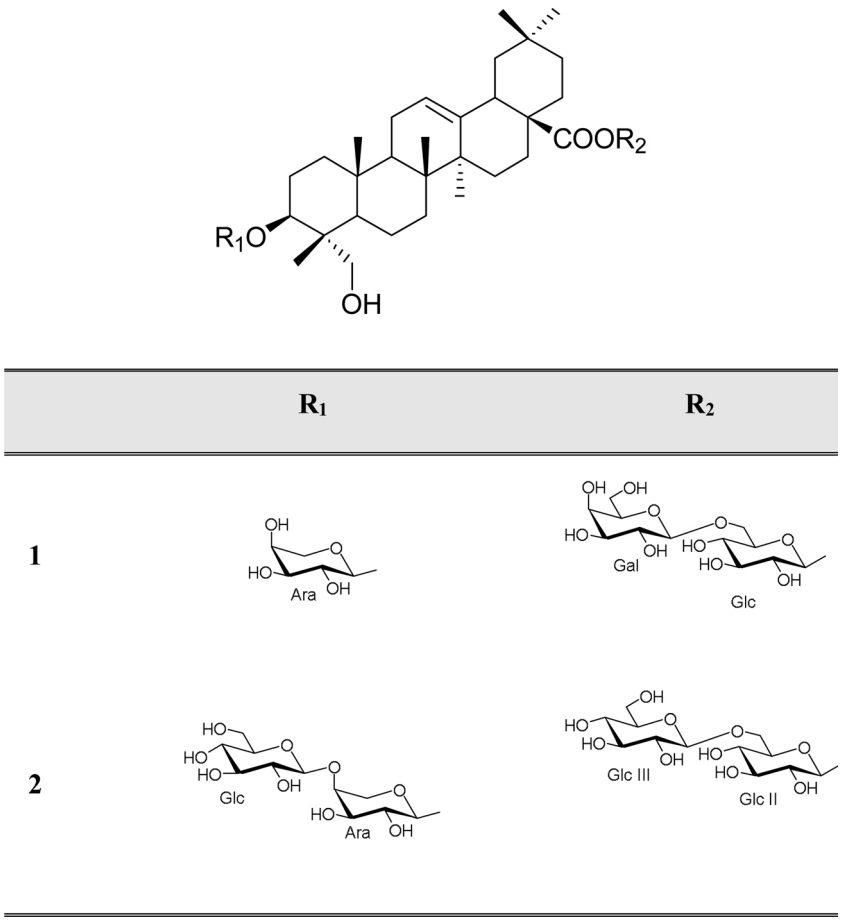

Fig. 1. The structures of compounds **1-2.**

28-*O*-*β*-D-galactopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**1**) and 3-*O*-*β*-D-glucopyranosyl-(1→4)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**2**). The thirteen known compounds, nine of them are saponins (**3-11**), two of them are iridoids (**12-13**), one of them is flavonoid glycoside (**14**) and finally last one is methyl glycoside (**15**), were identified by spectral methods including 1D-, 2D-NMR and HR-ESI/MS (high-resolution electrospray ionization mass spectrometry) techniques and some chemical examinations. Cytotoxic activities of compounds **1–2** and **1a-2a** were examined on different cells CD 34Lu, A549, CRL5807, CRL 5826, HTB-177 and HeLa by MTT method. Immunomodulatory activity of compounds **1–2** and **1a-2a** was also investigated by performing cytokine analysis with ELISA on saponins treated whole blood cells stimulated with PMA plus ionomycin.

2. Experimental

2.1. General experimental procedures

IR spectra were recorded using on an ATI Mattson 1000 Genesis series FT-IR instrument. Optical rotations were measured using a Rudolph Research Analytical Autopol I automatic polarimeter fitted with a sodium lamp. NMR measurements were achieved on a Varian AS 400 MHz and Varian ASP 600 MHz NMR spectrometers in DMSO-d_6. TMS was used as an internal standard and *J* values were reported in Hz. HR-ESI/MS was measured on a Bruker LC micro-Q-TOF mass spectrometer. MPLC was carried out on Buchi C-605 MPLC instrument. GC–MS analysis was performed by Agilent 789OB (GC) and 5977A (MSD) instrument with HP-5MS column (60 m, 0.25 mm, 0.25 u). For thin-layer chromatography, silica gel F_{254} (Merck 5554) and RP-18 F_{254s} (Merck 5560) pre-coated plates were used. Silica gel 60 (0.063−0.200 mm, Merck) and LiChroprep RP-18 (25−40 μm, Merck) were used both for column chromatography and MPLC. TLC plates were developed by spraying with 20 % H_2SO_4, followed by heating at 120 °C.

2.2. Plant material

Cephalaria tchihatchewii Hub.-Mor. & Matthews (Caprifoliaceae) was collected from Seferek Gateway in Mus-Varto about 1910 m in altitude, in August 2013, and identified by H. Sumbul and R. S. Gokturk (Akdeniz University, Faculty of Science, Department of Biology). A voucher specimen (No: R. S. Gokturk 7678) has been deposited at the Herbarium Research and Application Centre of Akdeniz University.

2.3. Extraction and isolation

The air-dried and powdered aerial parts of the plant (1.53 kg) were extracted with MeOH (5 × 4 L) overnight at room temperature, and the combined extracts were concentrated under vacuum at ~ 40 °C. The MeOH residue was extracted with *n*-BuOH-H_2O, 1:1 (8 × 50 mL) solvent system, and then the *n*-BuOH fraction was defatted with *n*-hexane (15 × 50 mL) to remove chlorophyll and oily substances. The re-purified *n*-BuOH fraction, which was found to be the most biologically active, was taken under investigation. A part of the *n*-BuOH fraction (66.7 g) was chromatographed on a RP silica gel *via* vacuum liquid chromatography (VLC) eluted with MeOH-H_2O solvent system (MeOH:H_2O, 0:100→100:0% by increasing polarity with MeOH 10 % gradient) to give 8 fractions. The combined fractions 4 and 5 (11.79 g) were loaded into the first medium pressure liquid chromatography (MPLC-1) (adsorbent: silica gel, column: 49 × 230, flow rate:25 mL/min, max pressure: 20 bar)

Table 1
^{1}H-, ^{13}C-NMR spectroscopic data for compounds **1-2**.[a,b,c,d]

position	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	38.5	0.84; 1.49; m	41.1	0.80; 1.45; m
2	25.6	1.05; 1.70; m	28.2	nd; 1.67; m
3	80.4	3.47; m	83.1	3.43; m
4	42.8	–	45.4	–
5	46.6	1.15; m	49.3	1.12; m
6	17.6	1.19; 1.40; m	20.3	1.11; 1.37; m
7	32.2	0.90; 1.16; m	34.9	1.10; 1.39; m
8	39.4	–	42.0	–
9	47.6	1.48; m	50.2	1.46; m
10	36.4	–	39.0	–
11	23.3	0.82; 1.80; m	26.1	nd; 1.76; m
12	122.1	5.14; s	124.7	5.13; s
13	144.0	–	146.6	–
14	41.8	–	44.4	–
15	27.6	0.94; 1.72; m	30.3	0.91; 1.67; m
16	22.9	1.58; 1.95; m	25.6	1.55; 1.93; m
17	46.4	–	49.1	–
18	41.2	2.70; m	43.8	2.70; m
19	46.0	1.08; 1.62; m	48.7	1.04; 1.60; m
20	30.7	–	33.4	–
21	33.7	1.14; 1.32; m	36.3	1.12; 1.32; m
22	32.0	nd; 1.54; m	34.7	1.08; 1.50; m
23	63.2	3.03; 3.36; m	65.8	3.06; 3.36; m
24	13.4	0.55; s	15.8	0.55; s
25	16.1	0.85; s	18.7	0.84; s
26	17.2	0.65; s	19.8	0.64; s
27	26.0	1.05; s	28.6	1.05; s
28	175.7	–	178.4	–
29	33.2	0.85; s	35.9	0.84; s
30	23.8	0.85; s	26.5	0.84; s
	Ara at C-3	**Ara at C-3**	**Ara at C-3**	**Ara at C-3**
1	105.2	4.17, brs	105.5	4.41, d (4.8)
2	71.5	3.28, m	74.3	3.53, m
3	73.2	3.27, m	74.4	3.77, m
4	68.0	3.57, m	81.9	3.58, m
5	65.5	3.28, 3.62, m	66.2	3.27, 3.62, m
	Glc at C-28	**Glc at C-28**	**Glc**	**Glc**
1	94.6	5.22, d (7.6)	106.8	4.30, d (7.8)
2	71.1	3.10, m	72.9	3.04, m
3	76.8	3.35, m	77.6	2.94, m
4	69.6	3.20, m	69.4	3.63, m
5	77.2	3.23, m	79.4	3.29, m
6	68.6	3.50, 3.90, m	64.0	3.44, 3.60, m
	Gal	**Gal**	**Glc II at C-28**	**Glc II at C-28**
1	101.6	4.45, d (7.6)	97.2	5.20, d (8.4)
2	72.8	3.08, m	75.4	3.07, m
3	71.7	3.80, m	79.5	3.20, m
4	67.9	3.25, m	72.2	3.20, m
5	74.8	3.43, m	79.9	3.04, m
6	61.8	3.40, 3.60, m	71.2	3.50, 3.90, m
			Glc III	**Glc III**
1			104.2	4.43, d (7.8)
2			73.7	3.09, m
3			77.4	3.43, m
4			70.5	3.25, m
5			79.4	3.11, m
6			64.4	3.39, 3.58, m

[a] ^{1}H -NMR and ^{13}C-NMR (δ) data were measured in DMSO-d_6 at 600 MHz. (δ) and 150 MHz, respectively.
[b] The assignments are based on COSY, HSQC and HMBC experiments.
[c] Coupling constants (J) in Hz are given in parentheses.
[d] nd: not determined.

with solvent systems $CHCl_3$-MeOH-H_2O (90:10:1; 80:20:2; 70:30:3; 61:32:7) and MeOH, to give 14 fractions. The 9th of these fractions (900 mg) was purified on a silica gel column eluted with $CHCl_3$-MeOH-H_2O (80:20:2) to give the newly described compound **1** (100 mg), and the known compound **6** (100 mg). The other new compound **2** (56.8 mg) was obtained by silica gel column chromatography eluted with the $CHCl_3$-MeOH-H_2O (70:30:3) solvent system from the 14th fraction along with the known compound **10** (105.4 mg). Fraction 6 (528.4 mg) was purified by silica gel CC using solvent system $CHCl_3$- MeOH-H_2O (80:20:2) to isolate the known compound **3** (108 mg). Fraction 4 (227.1 mg) was re-chromatographed on a silica gel column using solvent system $CHCl_3$-MeOH-H_2O (90:10:1) to yield the two known compounds **4** (54.2 mg) and **12** (21.3 mg). Fraction 7 (270 mg) was further separated by an open silica gel column chromatography eluting with the $CHCl_3$-MeOH-H_2O (90 :10: 1) solvent system to give compounds **5** (23 mg), and **11** (12.3 mg). Compound **7** (60 mg) was purified from 8th fraction of VLC (415.9 mg) by the open silica gel column chromatography with $CHCl_3$-MeOH-H_2O (70:30:3) solvent system as mobile phase. The combination of fractions 11 and 12 (1.1 g) was separated by a silica gel column chromatography with the solvent system $CHCl_3$-MeOH-H_2O (80:20: 2) in order to purify compounds **8** (126 mg) and **9** (50 mg), respectively. The 2nd fraction of VLC (16 g) was subjected to MPLC-2 [adsorbent: RP silica gel, column: 36 × 460 mm, flow rate: 20 mL/min, max. pressure: 30 bar], eluted with MeOH-H_2O solvent system to give 15 fractions. Fractions 2 (549 mg) and 4 (280 mg) were separately loaded to different open silica gel column chromatography with $CHCl_3$-MeOH-H_2O (70:30:3) solvent system and gave compounds **15** (216 mg) and **13** (52 mg), respectively. The last isolation process MPLC-3 [adsorbent: silica gel, column: 36 × 460 mm, solvent system $CHCl_3$-MeOH-H_2O 90:10:1; 80:20:2; 70:30:3; 61:32:7 (1 L each), flow rate: 25 mL/min, max. pressure: 30 bar] was carried out on the combined fractions 11 and 12 (1.435 g) of MPLC-2, and gave total of 16 fractions. The fractions 9 and 10 (72 mg) were combined and fractionated by a RP silica gel column chromatography with MeOH-H_2O (6:4) solvent system and yielded the last compound **14** (10 mg).

2.3.1. *Tchihatchewoside A (1)*

White, amorphous powder; $[\alpha]^{27}_{D}$ –22.2 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3401.44, 2922.75, 1732.32, 1640.29, 1464.54, 1388.03, 1261.59, 1075.98 cm^{-1}; ^{1}H -NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz) see Table 1; HR-ESI/MS *m/z* 951.49243 $[M + Na]^+$ (calcd for $C_{47}H_{76}O_{18}$ Na, 951.49239).

2.3.2. *Tchihatchewoside B (2)*

White, amorphous powder; $[\alpha]^{27}_{D}$ -6.06 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3400.96, 2942.74, 1738.56, 1642.62, 1461.95, 1387.84, 1261.42, 1076.87 cm^{-1}; ^{1}H- NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz) see Table 1; HR-ESI/MS *m/z* 1091.56453 $[M+H]^+$ (calcd for $C_{53}H_{87}O_{23}$, 1091.56327).

2.4. *Alkaline hydrolysis*

Pure compounds **1–2** (3−5 mg) were subjected to alkaline hydrolysis according to the literature. (Sarikahya and Kirmizigul, 2010). Alkaline hydrolysis of **1** and **2** afforded known triterpene glycosides, leontosides A and B (Mzhel'skaya and Abubakirov, 1967), respectively.

2.5. *Sugar analysis*

The identification of sugar moieties of the newly described saponins was performed by GC–MS analysis following the procedure reported by Sarikahya and Kirmizigul (2010). L-Arabinose, D-xylose, D-galactose and D-glucose were detected by co-injection of the hydrolysate with standard silylated sugars peaks at tR (min) 17.99, 19.71, 20.93 and 21.41, respectively. Identification of L-arabinose, D-galactose and D-glucose was detected for compound 1, by recording peaks respectively at 18.10, 20.87 and 21.32 min, while L-arabinose and D-glucose for compound 2, were respectively identified by peaks at 18.10 and 21.32 min.

2.6. *Cytotoxic activity assay*

According to literature (Mossman, 1990), cytotoxicity of selected saponins were analyzed against CCD 34Lu (normal lung fibroblast cells,), HeLa (human cervix adenocarcinoma), A-549 (human alveolar

Table 2
Cytotoxic activity results for compounds **1-2** and **1a-2a** by MTT assay.

Compounds	Cell Lines IC_{50} (μM)					
	CCD 34Lu	A549	CRL 5807	CRL 5826	HTB-177	HeLa
1	–	–	–	–	–	–
2	–	–	–	–	–	–
1a	18.35 ± 2.5	>50	>50	25.51 ± 3.73	>50	89 >50
2a	–	–	–	–	–	–
Doxorubicin	14.7 ± 4.7	16.97±1.71	0.35±2.91	10.42 ± 1.41	18.41±0.03	8.84±1.62

- not determined.

adenocarcinoma), CRL5807 (bronchioalveolar carcinoma; non-small cell lung cancer), CRL 5826 (squamous cell carcinoma; mesothelioma) and HTB-177 (carcinoma; large cell lung cancer) cancer cells using MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide)] assay. The viability (%) was determined by the following formula:

% Viable cells = [(absorbance of treated cells - (absorbance of blank)]/ [(absorbance of control - (absorbance of blank)] x 100

2.7. Immunomodulatory activity assay

Human whole blood was suspended in RPMI-1640 medium supplemented with 10 % FBS, 100 U/mL penicillin and 100 mg/mL streptomycin in a 1:10 ratio. PMA (50 ng/mL) and ionomycin (400 ng/mL) were added to the whole blood for stimulation of immune system cells. The samples were dissolved in DMSO which was used as negative control. One ml of the blood treated with PMA (50 ng/mL) and ionomycin (400 ng/mL) were added into each well of 24-well plate and incubated at 37 °C for 24 h in the absence and presence of 3 μg/mL concentrations of the samples. Then, the culture supernatants were evaluated using the specific ELISA method for IL-4, IFN-γ and IL-1β (Top et al., 2017). The protocol was approved by the Human Ethics Committee of Ege University, and all of the procedures used conformed to the declaration of Helsinki. The subjects were informed about the procedures and signed the informed consent forms (approval number: E.215344).

3. Results and discussion

In the current work, the isolation and structure elucidation of two newly described bisdesmosidic oleanane-type triterpenoid saponins (**1–2**), named tchihatchewosides A–B [Fig. 1] were reported from the aerial parts of *C. tchihatchewii*. Furthermore, thirteen known compounds nine of them are saponins namely; hedera nepalensis saponin F (**3**) (Kizu et al., 1985), α-hederin (**4**) (Aliev and Movsumov, 1976), dipsacus saponin A (**5**) (Jung et al., 1993), akebia saponin D (**6**) (Mukhamedziev et al., 1971), 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester (**7**) (Kawai et al., 1988), elmalienoside B (**8**) (Sarikahya and Kirmizigul, 2012), dipsacoside B (**9**) (Mukhamedziev et al., 1971), akebia saponin F (**10**) (Higuchi and Kawasaki, 1972), cauloside C (**11**) (Strigina et al., 1970) two of them are iridoids namely; loganin (**12**) and loganic acid (**13**) (Calis et al., 1984) one flavonoid namely; luteolin-7-*O*-glycoside (**14**) (Markham et al., 1978) and finally one methyl glycoside (**15**) (Rutherford et al., 1986) were obtained. The structures of all compounds (**1-15**) (Figure 13S, Sup. Info.) were elucidated on the basis of their chemical and spectroscopic data.

Compound **1** was obtained as an amorphous powder. The HR-ESI/MS (positive-ion mode) of **1** exhibited a sodiated molecular ion, $C_{47}H_{76}O_{18}Na$ at *m/z* 951.49243 $[M + Na]^+$ (theoretical *m/z* 951.49239). This was confirmed and supported by the NMR spectroscopic data for this compound [Table 1]. The IR spectrum of **1** showed absorptions for olefin (1640.29 cm^{-1}), hydroxyl (3401.44 cm^{-1}), and ester (1732.32, 1261.59 cm^{-1}) functionalities. The 1D- NMR data of **1** revealed the presence of six methyl groups at δ 1.05 (H_3-27), 0.85 (H_3-25), 0.85 (H_3-29), 0.85 (H_3-30), 0.65 (H_3-26), and 0.55 (H_3-24) as a singlet, one oxygen bearing methine proton at δ 3.47 (H_1-3, m), a hydroxymethyl protons at δ 3.03, 3.36 (H_2-23, m), one olefinic proton at δ 5.14 (H_1-12, brs) with two typical olefinic carbon signals at δ_C 122.1 and 144.0, a hydroxymethyl carbon at δ_C 63.2 (C-23), an oxygen-bearing methine carbon at δ_C 80.4 (C-3) and an ester carbonyl group at δ_C 175.7 (C-28) indicating hederagenin as an aglycone which is also proved with literature findings (Lin et al., 1998). The sugar part of **1** was found to consist of three anomeric proton signals, for three sugar moieties that resonated at δ_H 5.22 (d, *J* =7.6 Hz), 4.45 (d, *J* =7.6 Hz), and 4.17 (brs) in the ^{1}H-NMR spectrum. The coupling constants confirmed the β-glycosidic linkages for two sugar units including one glucose and one galactose units and α-glycosidic linkage for one arabinose unit. The GC–MS analysis of silly derivative of **1** also confirmed the types of sugar units, accurately (see Exp. Section). The HSQC spectrum of **1** helped clarify the proton-carbon correlations of the compound (Sup. Info.). After alkaline hydrolysis of **1**, one glucose and one galactose units were determined in the aqueous phase of the hydrolysate by GC–MS analysis. According to the ^{1}H-NMR data, only one arabinose unit remained in the structure of prosapogenin **1a** which was obtained after alkaline hydrolysis. All these findings indicated that only one arabinose moiety is affixed at the C-3 position of the aglycon through an etheric linkage. In the HMBC spectrum of **1**, specific correlations were observed between signals at δ_H 4.17 (H-1 of Ara) and δ_C 80.4 (C-3 of the aglycon), δ_H 5.22 (H-1 of Glc) and δ_C 175.7 (C-28 of the aglycon), and δ_H 4.45 (H-1 of Gal) and δ_C 68.6 (C-6 of Glc) showed the linkage points of the sugar moieties to the aglycon and to each other, respectively. Hence, the structure of this glycoside was established as 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl ester,] namely tchihatchewoside A (**1**) [Fig. 1].

Compound **2** was yielded as colorless amorphous powder. Its molecular formula, $C_{53}H_{87}O_{23}$, was calculated on the basis of its molecular ion at the positive-ion HR-ESI/MS at *m/z* 1091.56453 $[M+H]^+$ (theoretical *m/z* 1091.56327 $[M+H]^+$) together with the ^{1}H- and ^{13}C-NMR spectra. A carbonyl, hydroxyl and olefinic characteristic absorptions could be noted in the IR spectra at 1738.56, 3400.96 and 1642.62 ppm, respectively. Analysis of the NMR data indicated that compound **2** is a hederagenin type triterpene saponin like compound **1** with four sugars that were identified as glucose and arabinose through GC–MS analysis results. Alkaline hydrolysis also indicated that compound **2** is a bisdesmosidic triterpenoid glycoside with two glucose moieties linked to the C-28 position of the aglycon, with one glucose and one arabinose moieties linked to the C-3 position of the aglycon. The linkage points of the sugar units to each other and to C-3 of aglycon was established from the HMBC correlations (Sup. Info.) of signals at δ_H 4.41 (H-1 of Ara) with δ_C 83.1 (C-3 of the aglycon), and δ_H 4.30 (H-1 of Glc) with δ_C 81.9 (C-4 of Ara). The sugar sequence at C-28 was determined by the following HMBC correlations: δ_H 5.20 (H-1 of Glc II) with δ_C 178.4 (C-28 of the aglycon) and δ_H 4.43 (H-1 of Glc III) with δ_C 71.2 (C-6 of Glc II). Accordingly, the structure of **2** was assigned as 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, namely tchihatchewoside B.

The organic layers of the alkaline hydrolysis of the pure compounds

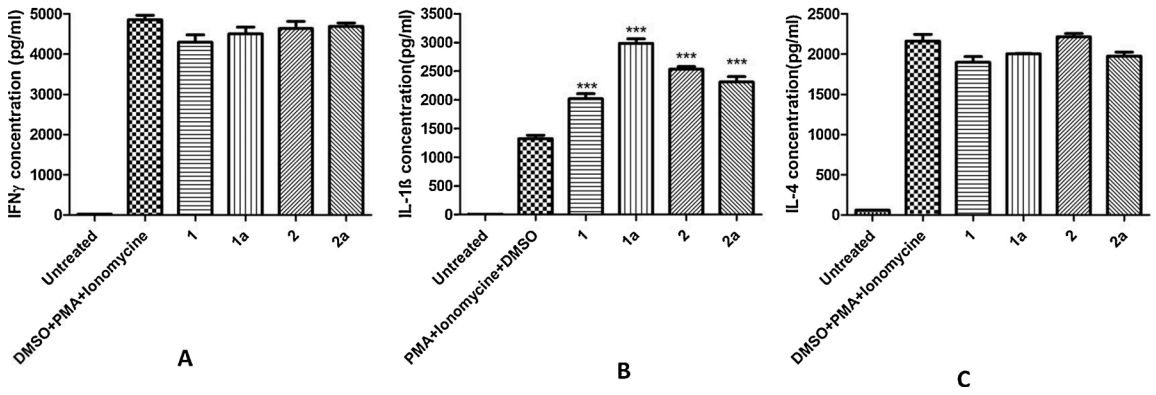

Fig. 2. Immunomodulatory activity results for compounds **1-2** and **1a-2a.** Immunomodulatory activity was carried out with PMA plus ionomycin in stimulated whole blood cells treated with saponins. The supernatant was analyzed for IL-4, IFN-γ and IL-1β cytokines by ELISA. **(A)** IFN-γ **(B)** IL-1β **(C)** IL-4*** $p < 0.001$.

1–2 afforded known triterpene glycosides, compound **1a**, leontoside A and compound **2a**, leontoside B (Mzhel'skaya and Abubakirov, 1967), respectively.

The cytotoxic effects of compounds **1–2** and prosapogenins **1a-2a** were examined using the MTT method [Table 2] with doxorubicin as positive control. According to these results, it was found that only compound **1a**, obtained alkaline hydrolysis of compound **1**, has a cytotoxic effect. Compound **1a** exhibited moderate cytotoxicity with IC_{50} value of 25.51 ± 3.73 μM against CRL 5826 lung carcinoma cells, compared to the IC_{50} value 10.42 ± 1.41 μM of doxorubicin. The cytotoxicity results are parallel to our previous studies, where the monodesmosidic hederagenin saponins are active against A-549 and HeLa cells using the MTT method. In this study davisianoside B, an oleanane type monodesmosidic saponin, showed significant activity against the lung cancer cell line A-549, even higher than standard doxorubicin (Sarikahya et al., 2018). Many isolation studies in literature supported that the cytotoxic activities of monodesmosidic saponins are higher than those of bisdesmosidic ones (Lacaille-Dubois, H. Wagner, 1996; Top et al., 2017). In these circumstances, the obtained results suggested that alkaline hydrolysis of bisdesmosidic saponins is a good method to the improve cytotoxicity.

The strong hemolytic activity of monodesmosidic triterpenoid saponins was also confirmed and reported in different studies (Gauthier et al., 2009; Sarikahya et al., 2018; Wang et al., 2007). This situation prevents the usage of saponins in the clinics. There are many different researches in the literature showing that this effect can be overcome by converting saponins into stable nanoparticles (Hu et al., 2010; Rejinold et al., 2011). Briefly, preparing nanoparticles contain saponin or encapsulating the saponin can be success to achieve its hemolytic effect. Thus, the saponins with strong cytotoxic effects might be applicable in targeted delivery system.

Regarding the immunomodulatory activity of compounds in whole blood cells stimulated with PMA plus ionomycin compared to the control group, new compounds **1–2** and prosapogenins **1a-2a** significantly increased IL-1β secretion (*** $p < 0.001$) [Fig. 2]. The results showed that hederagenin saponins have the potential to activate the innate immune response by stimulating the IL-1β cytokine, which plays an important role in the activation process of innate immunity. In our previous study, bisdesmosidic saponins with hederagenin aglycone showed remarkable IL-1β release (Sarikahya et al., 2018). Once again it is obvious that the type of aglycone and number of sugars are effective on immunomodulatory activity (Dinarello, 2018; Lacaille-Dubois, 2019).

4. Conclusion

This work evaluated the cytotoxic and immunomodulatory activities of two newly described oleanane-type saponins (**1–2**) named tchihatchewosides A–B and prosapogenins **1a-2a**. While the only compound **1a**, obtained alkaline hydrolysis of compound **1**, has a cytotoxic effect, new compounds **1–2** and prosapogenins **1a-2a** significantly increased IL-1β secretion. Thus, the present work supports the value of chemical and pharmacological investigations of *Cephalaria tchihatchewii* in the search for new anticancer and immunomodulatory agent.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2020.12.007.

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