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# Immunomodulatory, hemolytic properties and cytotoxic activity potent of triterpenoid saponins from *Cephalaria balansae*

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ABSTRACT

Phytochemical investigations on n-butanol extract of *Cephalaria balansae* Raus. (Caprifoliaceae) led to the isolation of four previously undescribed triterpenoid saponins based on hederagenin type aglycone, namely, balansoides A-D, along with ten known compounds. Their structures were proposed based on 1D and 2D NMR spectroscopic data, HRESIMS analysis and chemical evidence as 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin, 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester, 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-galactopyranosyl-(1 → 6)-β-D-glucopyranosyl ester and 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester. The four saponins were evaluated for their potential cytotoxic activity against HEK-293, A-549, HeLa cells and for hemolytic properties on human blood cells. Balansoide A displayed significant inhibitory effects on cancerous A-549 and HeLa cells, and non-cancerous HEK-293 cells with $IC_{50}$ values of 12, 15 and 8 μM, respectively. Balansoides A-D together with *n*-butanol extract exhibited considerable hemolysis in human erythrocyte cells. Immunomodulatory properties of balansoides A-D were also evaluated in activated whole blood cells by PMA plus ionomycin. While balansoides A-C increased IL-1β concentration with values of 1004.47, 991.57 and 966.50 pg/ml, only balansoide B augmented a slight IFN-γ secretion with value of 5219.14 pg/ml. None of the compounds changed IL-2 levels significantly.



## 1. Introduction

Saponins constitute a structurally highly diverse class of natural plant defense compounds. They are widely distributed in higher plants but are also found in some bacteria and some lower animal sources, like e.g. marine invertebrates (Netala et al., 2015). They are responsible for most of the observed biological effects such as antifungal, insecticidal, anthelmintic, cytotoxic, anti-inflammatory, immunostimulant (Francis et al., 2002; Lacaille-Dubois and Wagner, 1996; Kräutler et al., 2008; Sparg et al., 2004). Especially in recent years, researches on saponins have been directed on the immunotherapy methods *in vivo* and *in vitro* conditions (Nalbantsoy et al., 2012; Rajput et al., 2007). The presence of saponins has been reported in more than 100 families of plants (Hostettmann and Marston, 1995). The genus *Cephalaria* (Schrad. ex Roem. & Schult.) (Caprifoliaceae), which is one of these plants, has been used in traditional medicine for many years due to its antimicrobial, antifungal, cytotoxic, antioxidant, antidiabetic and hypothermic activities (Mbhele et al., 2015; Mustafayeva et al., 2010; Pasi et al., 2009; Podolak et al., 2010; Sarikahya et al., 2011) Many hederagenin type saponins have been isolated from *Cephalaria* species especially by our research group and other scientists (Godevac et al., 2010; Gulcemal et al., 2014; Kirmizigul et al., 1995; Pasi et al., 2009; Sarikahya et al., 2011; Sarikahya and Kirmizigul, 2012) As well as saponins, *Cephalaria* taxa involve some specialised metabolites such as iridoids, flavonoids, alkaloids, and lignans (Mojab et al., 2003; Movsumov et al., 2009; Mustafayeva et al., 2010; Sarikahya and Kirmizigul, 2010).

Preliminary TLC analysis of the *n*-BuOH extracts of *C. balansae* Raus. (Davis, 1972) suggest that it contains numerous triterpene

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glycosides which prompts us to make a phytochemical examination of this fraction. As a result, four previously undescribed hederagenin type triterpene glycosides (**1**–**4**) named balansoides A-D together with ten known natural compounds were isolated from *C. balansae*. The structures of these compounds were identified by chemical methods including acidic and alkaline hydrolysis, sillylation and extensive spectroscopic analysis, along with 1D 2D NMR and HRESIMS data. The four previously undescribed triterpenoid saponins which were purified were tested for their cytotoxicity against HeLa (human cervix adenocarcinoma), A-549 (human alveolar adenocarcinoma), and a normal cell line HEK-293 (human embryonic kidney cells) by the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide)] method. Balansoides A-D and the *n*-butanol extract were evaluated for their hemolytic activity in human erythrocyte cells. Immunomodulatory properties of previously undescribed compounds were also tested in activated whole blood cells by PMA plus ionomycin.

## 2. Results and discussion

The *n*-butanol extract of the aerial parts of *Cephalaria balansae* was submitted to vacuum liquid chromatography (VLC) and fractionated by repeated medium pressure liquid chromatography (MPLC) and open column chromatography (CC) applications over normal and RP-18 silica gel, yielding four previously undescribed triterpenoid saponins called balansoides A-D (**1**–**4**) (see Fig. 1) and ten known ones. Eight compounds (**5**–**12**) were determined as known hederagenin type triterpene glycosides which can be classified as monodesmosidic and bisdesmosidic. The monodesmosidic ones were elucidated as α-hederin (**5**) (Aliev and Movsumov, 1976) and sapindoside B (**6**) (Chirva et al., 1969). The bisdesmosidic glycosides were 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester (**7**) (Kawai et al., 1988), decaisoside D (**8**), decaisoside E (**9**) (Kong et al., 1993), elmalienoside B (**10**), elmalienoside C (**11**) (Sarikahya and Kirmizigul, 2012) and dipsacoside B (**12**) (Mukhamedziev et al., 1971). The other two known compounds, **13** and **14**, were determined as oleanoic acid (Lin et al., 1998) and 3-*O*-β-D-sitosterolglycoside (Chirva et al., 1970), respectively by comparison of their physical and spectroscopic data with those reported in the literature. All isolated compounds were identified by comparison of the 1D, 2D NMR (1H, 13C, APT, COSY, HSQC, HMBC) and HRESIMS techniques and chemical methods with reference data. All compounds were isolated as amorphous powders and each monosaccharide was confirmed by extensive 2D NMR data and GC-MS analysis which compared them with authentic samples (Sarikahya and Kirmizigul, 2010).

Compound **1** (balansoide A) (see Fig. 1) has a molecular formula of $C_{52}H_{84}O_{20}Na$, determined by HRESIMS (*m/z* 1051.5399 $[M + Na]^+$). The IR spectrum of **1** indicated the presence of hydroxyl (3387 $cm^{-1}$) and carbonyl (1696 $cm^{-1}$) groups in the molecule. The 1H NMR spectrum of **1** showed characteristic singlets due to six quaternary methyl groups at $\delta_H$ 1.07, 0.85 (x 3), 0.70 and 0.57, a hydroxymethyl group at $\delta_H$ 3.10, 3.32 (m) and an olefinic proton at $\delta_H$ 5.13 (br s). The 13C NMR spectrum also revealed the signals for six quaternary carbons at $\delta_C$ 33.3, 26.0, 23.9, 17.4, 16.0 and 13.5; an oxygen-bearing methine carbon at $\delta_C$ 79.8, a hydroxymethyl group at $\delta_C$ 62.8, a set of olefinic carbons at $\delta_C$ 144.5 and 121.8 and one carbonyl carbon at $\delta_C$ 179.3 confirmed that the aglycone of **1** is a hederagenin (Sharma et al., 2013) (see Tables 1 and 2). The C-3 oxymethine carbon and C-28 carbonyl carbon were observed at $\delta_C$ 79.8 and 179.3, respectively, which suggests that compound **1** is a 3-monodesmoside of hederagenin. For the sugar moieties, the 1H NMR spectrum of **1** displayed four anomeric proton signals at $\delta_H$ 4.30 (d, $J = 6.4$ Hz), 4.30 (d, $J = 6.4$ Hz), 5.13 (br s), and 5.30 (br s),

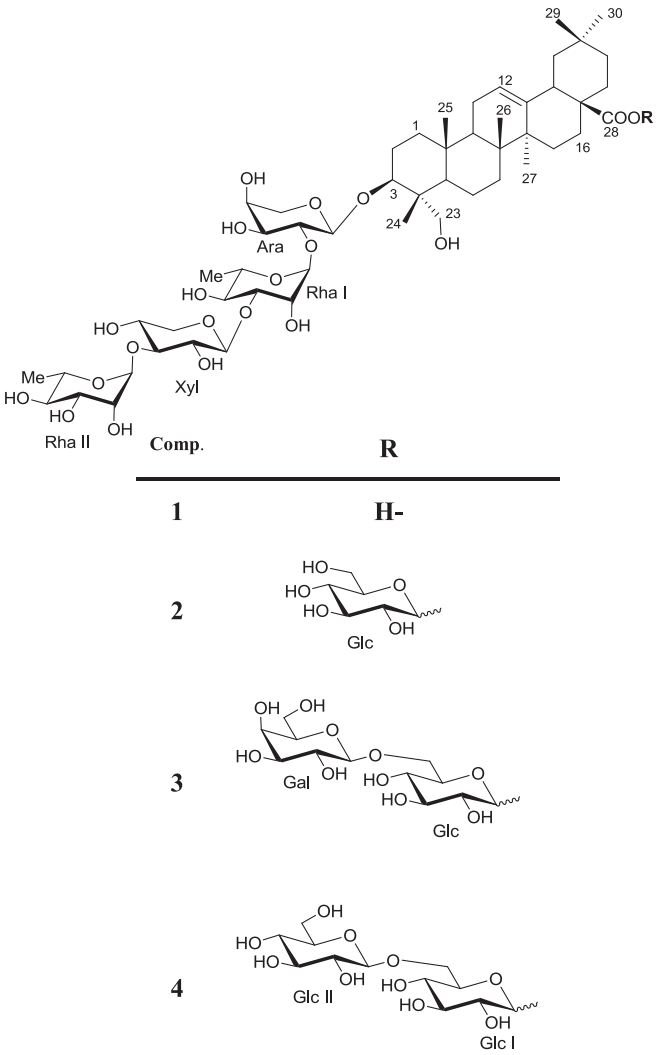


**Fig. 1.** Structures of compounds **1**–**4**.

giving in the HSQC spectrum cross-peaks with four anomeric carbon signals at $\delta_C$ 103.6, 105.9, 100.7 and 100.0, respectively. All proton signals for the sugar moieties were associated with COSY and HMQC spectra. While the acid hydrolysis of compound **1** gave L-arabinose, L-rhamnose, D-xylose, and hederagenin, alkaline hydrolysis showed no effect on compound **1**. The sugars obtained by aqueous acid hydrolysis of **1** were identified by GC-MS analysis which compared them with authentic samples (Sarikahya and Kirmizigul, 2010). These results were also confirmed by the HMBC data. The arabinose moiety was shown to be attached at C-3 of the aglycone by an observed HMBC correlation of $\delta_H/\delta_C$ 4.30 (d, $J = 6.4$ Hz, Ara H-1)/79.8 (Agly C-3). On the other hand, long-range correlations between the H-1 proton of L-rhamnose I at $\delta_H$ 5.30 and the C-2 carbon of L-arabinose at $\delta_C$ 74.0, the H-1 proton of D-xylose at $\delta_H$ 4.30 and the C-3 carbon of L-rhamnose I at $\delta_C$ 81.5 and the H-1 proton of L-rhamnose II at $\delta_H$ 5.13 and the C-3 carbon of D-xylose at $\delta_C$ 80.8 showed the linkaging points of the sugar molecules to one another. On the basis of the above results, the structure of compound **1** was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin.

Balansoide B (**2**) exhibited in the HRESIMS (positive-ion mode),

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