Research in Pharmaceutical Sciences, June 2024; 19(3): 347-355
Received: 26-04-2022
Peer Reviewed: 13-07-2022
Revised: 13-02-2023
Accepted: 01-06-2024
Published: 01-07-2024

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

*Original Article*

# Isolation of two steroidal saponins with antileishmanial activity from *Allium giganteum* L.

**Farnaz Chaparian, Zeinab Delazar, and Masoud Sadeghi Dinani***

*Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.*

## *Abstract*

**Background and purpose:** Alliums are rich sources of steroidal saponins, flavonoids, and sulphoric compounds of which steroidal saponins have recently received more attention due to their important pharmacological activities. *Allium giganteum* (giant onion) which is named locally "Couria" in the Northeast of Iran, is grown widely in "Kouh-Sorkh" mountains in Khorasan province.
**Experimental approach:** Phytochemical investigation of chloroform-methanol and aqueous extract of the plant resulted in the isolation and identification of two steroidal saponins, using comprehensive spectroscopic methods including 1D and 2D NMR and MS.
**Findings/Results:** The chemical structures of the isolated saponins were determined as (22S)-cholesta-1b,3b,16b,22b-tetraol 5-en, and 3-O-β-D-glucopyranosyl 26-O-β-D-glucopyranosside and (25R)-26-O-β-D-glucopyranosyl-5α-furostan-1α,3β,22α,26-tetraol3-O-{β-D-galactopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}. Investigation of *in vitro* antileishmanial activity of the isolated compounds at 10, 50, and 100 μg/mL exhibited significant leishmanicidal against the promastigotes of *Leishmania major*.
**Conclusion and implications:** The results established a valuable basis for further studies about *A. giganteum* and the anti-parasitic activity of steroidal saponins.

**Keywords:** *Allium giganteum*; Leishmania; Saponins; Structure elucidation.

## INTRODUCTION

Amaryllidaceae is a family of plants with 85 genera and approximately 1100 species which are mainly present in tropical and subtropical areas and dry regions (1). Although the origin of these plants is central Asia with a history of 3000 years of use, nowadays they are spread worldwide and among common edible and medicinal plants (2-5).

*Allium* is a genus that belongs to Amaryllidaceae which has 750 species (4). Iran is known as a main source of *Allium* species which are mainly found in the Zagros mountains and northeast regions of the country (2).

Historically, these plants have been used both for cooking and as raw vegetables, as well as medicinal plants for the treatment of different diseases like diabetes, hypercholesterolemia, blood hypertension, heart disease, insect bites, and tumors (6,7).

Alliums are the main sources of phytonutrients and have important secondary metabolites like steroidal saponins, sapogenins, flavonoids, and sulfur components (2,8), among them, steroidal saponins and sapogenins are some of the most important compounds in these genera (9,10).

Steroidal saponins in nature are found as glycosides which have features like frothing in water, hemolytic effect, being toxic for fishes, and complex formation with cholestrin (11).

*Corresponding author: M. Sadeghi Dinani
Tel: +98-3137927129, Fax: +98-3136680011
Email: m_sadeghi@pharm.mui.ac.ir

| Access this article online | |
|---|---|
| | **Website:** http://rps.mui.ac.ir |
| | **DOI:** 10.4103/RPS.RPS_71_21 |

There are numerous scientific reports about the various pharmacologic effects of saponins such as anti-tumor effect, anti-fungal effect, inhibitory effect on abnormal bleeding of the uterus, chronotropic effect, and protective effect on gastric ulcers (12). In addition, these compounds have a broad spectrum of biological activities like hemolytic, hypocholesterolemic, immune system modulating, anti-inflammatory, anti-ulcer, and leishmanicidal effects (13,14).

*Allium giganteum* (giant onion) which is named locally "Couria" in the Northeast of Iran, is grown widely in "Kouh-Sorkh" mountains in Khorasan province. The plant leaves are used locally as a condiment and also as raw vegetables for cooking bread and making dishes (15).

According to the importance of steroidal saponins and their biological effects and in continuous to our previous studies for completing the project of phytochemical investigation of *Allium* species of Iran, extraction, identification, and antileishmanial activity evaluation of steroidal saponins from the aqueous and chloroform-methanol extract of *A. giganteum* flowers was conducted in this study.

## MATERIALS AND METHODS

### *General experimental procedures*

Medium-pressure liquid chromatography (MPLC) was performed (Buchi Gradient System C-605 apparatus, Switzerland) using a glass column of LiChroprep® RP-18 (25-40µm). Thin-layer chromatography (TLC) was performed on $SiO_2$ plates with BuOH: $H_2O$: $CH_3COOH$ (60:25:15 v/v/v) (BAW) as a mobile phase and cerium sulfate in 2N $H_2SO_4$ as the reagent for visualizing the spots.

High-performance liquid chromatography (HPLC) was performed using Waters 515 apparatus equipped with a refractive index detector (Waters 2414) and UV detector (Waters 2487) (Waters, USA), using semipreparative C18 column (Novapak® 7.8 × 300 mm) and analytical C18 column (Novapak® 3.9 × 300 mm) in isocratic mode.

H and C nuclear magnetic resonance (NMR) spectra were recorded by Bruker 400 MHz (H at 400 MHz and C at 100 MHz) spectrometer (Bruker, Germany), using the solvent signal for calibration ($CD_3OD$: δH=3.31, δC=49.0). Distortionless enhancement by polarization transfer (DEPT) experiments was used to determine the multiplicities of CNMR resonances.

2D heteronuclear multiple bond correlation (HMBC), optimized for $^{2,3}$JCH of 8 Hz, was used for the determination of two and three-bond heteronuclear 1H-13C connectivities, while 2D heteronuclear single-quantum coherence (HSQC), interpulse delay set for $^{1}$JCH of 130 Hz, and correlated spectroscopy (COSY) were used for determination of one-bond heteronuclear 1H-13C connectivities and homoneuclear 1H-1H connectivities, respectively. Electrospray ionization mass spectra (ESIMS) were prepared by Shimadzu LCMS 2010 EV (Shimadzu, Japan), using methanol as the solvent.

### *Plant material*

The whole plant of *A. giganteum* was collected from "Kaashmar", 1065 meters high from sea level, Khorasan province, Iran during the Spring of 2019. A voucher specimen (No. 43213) was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

### *Isolation and purification*

Flowers of *A. giganteum* were separated, air-dried in the shade, and powdered employing a mill. The powder (835 g) was extracted at room temperature in a four-step extraction method with increasing solvent polarity using the solvents consisting of hexane, chloroform, chloroform-methanol (9:1), and methanol. Extraction was done using the maceration method, performing each step four times with 5 L of solvent under occasional stirring. Methanol extract of the sample was concentrated under vacuum, dissolved in water, and then by adding n-butanol, distributed between two solvents. The resulting butanol and aqueous layers were separated and concentrated under a vacuum.

The chloroform-methanol (9:1) extract of the sample was concentrated under vacuum, yielding a crude dried extract (15.32 g) which was fractionated by MPLC on an RP-18 column (36 × 460 mm) using a linear gradient solvent system of $H_2O$ to $CH_3OH$. Fractions were analyzed by TLC ($SiO_2$, BAW 60:15:25 v/v/v) and similar fractions were pooled together. Based on TLC and preliminary NMR analysis, 3rd fraction was considered to be richer in steroidal saponins, which was concentrated by rotary evaporator and subjected to HPLC for further purification. The final purification of the fraction was performed by HPLC using a semi-preparative C18 column (Novapak® 7.8 × 300 mm) and $H_2O$:$CH_3OH$ (80:20) mobile phase in isocratic mode, resulting in 10 mg of compound (1).

The aqueous extract of the sample was concentrated under vacuum, yielding a crude dried extract (12.97 g) which was fractionated by MPLC on an RP-18 column (36 × 460 mm) using a linear gradient solvent system of $H_2O$ to $CH_3OH$. Fractions were analyzed by TLC ($SiO_2$, BAW 60:15:25 v/v/v) and similar fractions were pooled together. Based on TLC and preliminary NMR analysis, the 8th fraction was considered to be richer in steroidal saponins, which were concentrated by a rotary evaporator and subjected to HPLC for further purification. The final purification of the fraction was performed by HPLC using a semi-preparative C18 column (Novapak® 7.8 × 300 mm) and $H_2O$:$CH_3OH$ (80:20) mobile phase in isocratic mode, resulting in 22 mg of compound (2).

***Evaluation of antileishmanial activity***

*Leishmania parasites*

Cryopreserved *Leishmania major* (MRHO/IR75/ER) were obtained from the Department of Parasitology & Mycology, Isfahan University of Medical Sciences and were transferred to modified Nicole Novy Neal (N.N.N.) medium supplemented with 4% brain heart infusion (BHI, 0.2 mL), streptomycin (100 µg/mL) and penicillin (100 U/mL). The promastigotes were then passaged in complemented RPMI 1640 with fetal calf serum (FCS, 10% v/v), L- glutamine (2 Mm), penicillin (100 U/mL), and streptomycin (100 µg/mL), and incubated at 25 °C. The antileishmanial activity was evaluated using promastigotes in the logarithmic phase.

*Antileishmanial assay*

The antileishmanial assay was performed as described by Kazemi Oskuee *et al.* (16). Briefly, *L. major* promastigotes 4 × $10^5$ in 400 µL complemented RPMI were cultured in 24-well plates. The steroidal saponin was dissolved in RPMI 1640 with the aid of 2% DMSO as co-solvent and added to the wells to make the final concentrations of 10, 50, and 100 µg/mL. The plates were incubated at 25 °C for 2 days and the number of viable parasites was counted on the periods of 12, 24, and 48 h. Amphotericin B at 0.5 and 1 µg/well and RPMI medium were used as the positive and negative control groups, respectively.

***Statistical analysis***

Antileishmanial activities were reported as mean ± SD and statically analyzed by one-way ANOVA followed by the Tukey-Kramer post-hoc test using SPSS Ver. 16. The *P*-values < 0.05 were considered statistically significant.

## RESULTS

Based on TLC and preliminary NMR screening, two saponins-rich fractions of the plant extracts were selected for further purification, resulting in the isolation and identification of 2 steroidal saponins (compounds 1 and 2; Fig. 1). The chemical structure of isolated compounds was determined using comprehensive spectroscopic methods and also by comparison of the spectral data with those reported in the literature.

***Characterization of compound (1)***

Compound (1) was isolated as an amorphous solid and showed in the positive ESIMS spectrum a pseudomolecular ion peak at m/z 781.4 [M+Na]+ which together with CNMR data indicated the molecular formula $C_{39}H_{66}O_{14}$. Diagnostic resonances of the HNMR spectrum were those attributed to two tertiary methyls (2 singlets: δH 0.78 (1H) and 0.95 (1H); CNMR: δC 13.81 and 14.81), two

overlapped secondary methyl (2 doublets: δH 0.77 (1H, d, 6.4) and 0.79 (1H, d, 6); CNMR: δC 23.26 and 12.16), one olefinic δH 5.42 (1H, d, 4.8), and two anomeric protons δH 4.03 (1H, d, 7.23) and 4.22 (1H, d, 7.64). The CNMR spectrum showed 35 resonance lines, supporting the molecular formula deduced from ESIMS, 27 of them were attributed to the aglycone part and 12 to two hexose monosaccharides. Diagnostic resonances regarding the glycone part were anomeric carbon resonances at δC 100.93 and 107.31. Other diagnostic resonances in the CNMR spectrum of compound (1) were δC 70.09 which was attributed to the C-22, δC 83.24 for C1 (C-OH), δC 139.55 for C5 (C=C), δC 126.2 for C6 (C=C), and δC 83.45 for C16 (C-OH) of aglycon part.

Determining the glycon part of the compound (1), using the first anomeric proton position (H1I; δH 4.03) and HSQC and COSY spectral data, especially the characteristic large coupling constant of H4I and H1I, the two sugars were determined as β-D-glucopyranoside. This was confirmed by the glycosylation shifts of C2I and C4I and also by the fragmentation peaks in the ESIMS spectrum due to the loss of sugar units from the pseudomolecular ion.

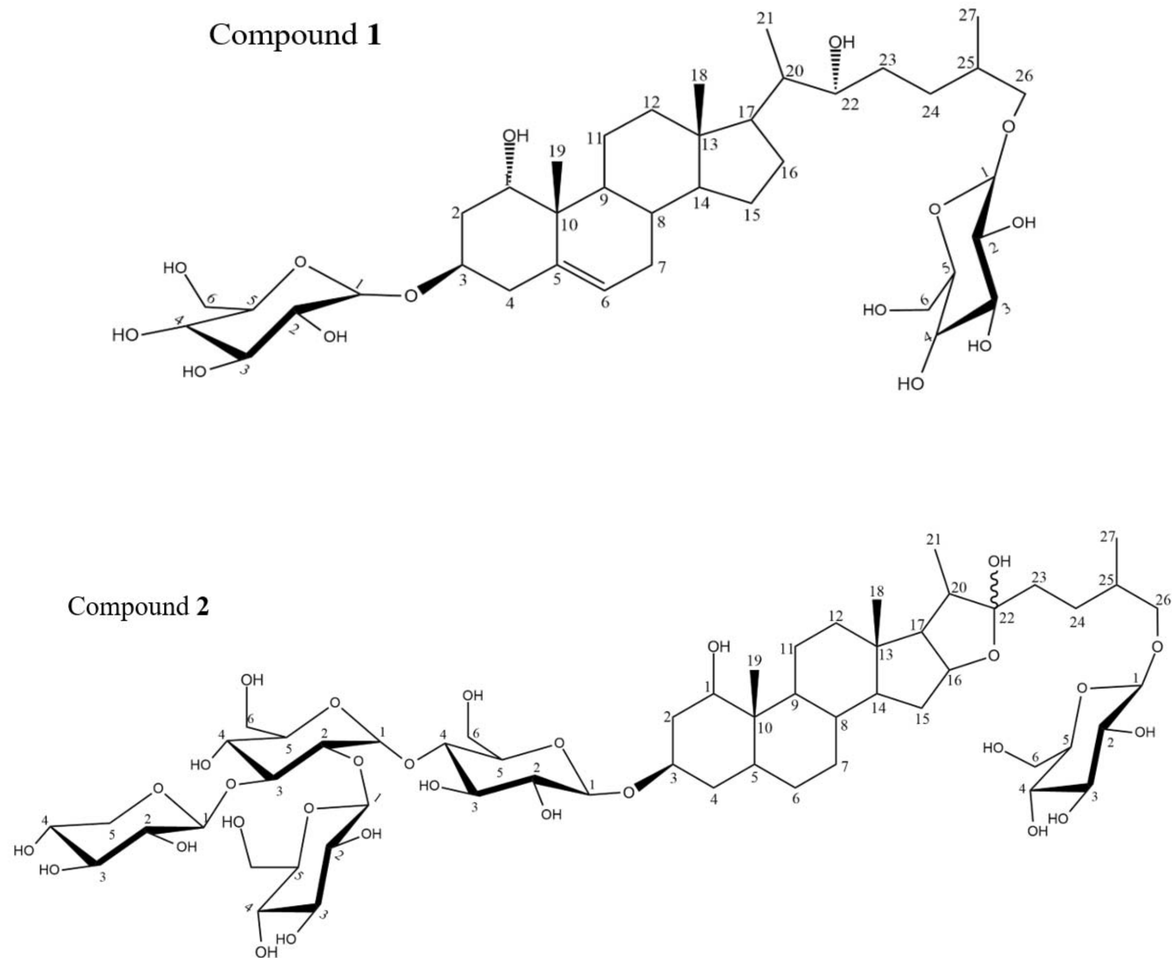


**Fig. 1.** Chemical structure of compounds **1** and **2.**

***Characterization of compound (2)***

The saponin nature of compound (2) was confirmed by 1H and 13CNMR spectra of the compound and the existence of diagnostic and characteristic signals of saponins especially two tertiary methyls (2 singlets: δH 0.75 (1H) and 0.94 (1H); CNMR: δC 16.25 and 17.34), two secondary methyls (2 doublets; δH 0.85 (1H,d, 6.8), 0.90 (1H, d, 6.8); CNMR: δC 16.25, 17.08), five anomeric protons (δH 4.15, 4.31, 4.49, 4.51, 4.85) and related anomeric carbon signals (δC 102.60, 104.26, 104.57, 104.72, and 104.93) (Tables 1 and 2). In the ESIMS spectra, compound (2) showed a pseudomolecular ion peak at m/z 1231.33 [M+Na]+ in the positive-ion mode which together with the CNMR data, suggested its molecular formula as $C_{56}H_{94}O_{29}$.

According to H and CNMR spectra of compound 2, two hydroxyl groups were also implied from the molecular formula which were determined to be placed at C1(δC 77.89) and C22 (δC 114.00).

To deduce the glycon part of (2), starting from the first anomeric proton (H1I; δH 4.15) and using the HSQC and COSY spectral data, the sugar chain was determined to be composed of two β-D-glucopyranoside, two β-D-galactopyranoside, and β-D-xylopyranoside monosaccharides, completing the sugar chain structure elucidation (Table 2). This was further confirmed by the fragmentation peaks in the ESIMS spectrum due to the loss of sugar units from the pseudomolecular ion.

**Table 1.** $^{1}$HNMR and $^{13}$CNMR data of the aglycon part of compounds **1** and **2** (400 MHz, $CD_3OD$)

| **Position** | **Compound 1** | | **Compound 2** | |
|---|---|---|---|---|
| | **$\delta_C$ (mult.)** | **$\delta_H$ (int., mult., *J* in Hz)** | **$\delta_C$ (mult.)** | **$\delta_H$ (int., mult., J in Hz)** |
| **1a** | 83.24 | 3.59 | 77.89 | 0.94 |
| **1b** | - | - | - | 1.73 |
| **2a** | 37.10 | 1.80 | 30.47 | 1.32 |
| **2b** | - | - | - | 1.51 |
| **3** | 69.09 | 3.75 | 87.88 | 3.43 |
| **4a** | 41.47 | 2.50 | 39.78 | 2.48 |
| **4b** | - | 2.58 | - | 2.58 |
| **5** | 139.55 | - | 34.97 | - |
| **6** | 126.2 | 5.42 | 41.18 | - |
| **7a** | 32.51 | 1.31 | 32.85 | 1.73 |
| **7b** | - | 1.73 | - | 1.87 |
| **8** | 34.18 | 1.27 | 27.77 | 1.54 |
| **9** | 51.21 | 1.16 | 55.68 | 0.86 |
| **10** | 43.27 | - | 36.86 | - |
| **11a** | 24.69 | 2.41 | 21.98 | 1.46 |
| **11b** | - | 1.47 | - | 1.39 |
| **12a** | 43.26 | 2.09 | 40.78 | 1.63 |
| **12b** | - | 1.39 | - | 1.04 |
| **13** | 42.85 | - | 42.22 | - |
| **14** | 56.21 | 0.95 | 57.26 | 1.04 |
| **15a** | 37.78 | 1.69 | 32.75 | 1.18 |
| **15b** | - | 2.22 | - | 2.08 |
| **16** | 83.45 | 4.51 | 82.46 | 4.49 |
| **17** | 58.84 | 1.91 | 63.09 | 1.63 |
| **18** | 13.81 | 0.78 | 16.25 | 0.75 |
| **19** | 14.81 | 0.95 | 17.34 | 0.94 |
| **20** | 36.31 | 2.42 | 41.22 | 1.87 |
| **21** | 12.16 | 0.79 | 16.25 | 0.85 |
| **22** | 70.09 | - | 114.00 | - |
| **23a** | 37.07 | 1.73 | 31.38 | 1.70 |
| **23b** | - | 1.64 | - | 1.46 |
| **24a** | 34.18 | 1.78 | 28.93 | 1.50 |
| **24b** | - | 1.55 | 76.00 | 1.27 |
| **25** | 29.78 | 1.44 | 31.37 | 1.18 |
| **26a** | 73.49 | 0.78 | 76.00 | 3.27 |
| **26b** | - | - | - | 3.21 |
| **27** | 23.26 | 0.77 | 17.08 | 0.90 |

NMR, Nuclear magnetic resonance.

**Table 2.** $^{1}$HNMR and $^{13}$CNMR data of sugar part of the compound (**1**) and (**2**) (400 MHz, 100 MHz; $CD_3OD$)

| Position | 2 $\delta_H$ (int, mult, J in Hz) | $\delta_C$ (mult) | 1 $\delta_H$ (int, mult, J in Hz) | $\delta_C$ (mult) |
|---|---|---|---|---|
| **I** | **Glucopyranoside 1** | | **Glucopyranoside 1** | |
| 1 | 4.15 | 102.60 (CH) | 4.03 | 100.93 |
| 2 | 3.19-3.79 | 75.31 (CH) | 3.61 | 74.36 |
| 3 | 3.19-3.79 | 79.80 (CH) | 3.59 | 77.78 |
| 4 | 3.19-3.79 | 70.96 (CH) | 4.21 | 72.34 |
| 5 | 3.19-3.79 | 77,45 (CH) | 3.43 | 75.33 |
| 6 | 3.19-3.79 | 61.10 ($CH_2$) | 3.23 | 62.22 |
| **II** | **Glucopyranoside 2** | | **Glucopyranoside 2** | |
| 1 | 4.31 | 104.26 (CH) | 4.22 | 107.31 |
| 2 | 3.19-3.79 | 75.60 (CH) | 3.75 | 75.33 |
| 3 | 3.19-3.79 | 80.20 (CH) | 3.45 | 78.11 |
| 4 | 3.19-3.79 | 71.54 (CH) | 4.02 | 72.90 |
| 5 | 3.19-3.79 | 78.12 (CH) | 3.56 | 76.27 |
| 6 | 3.19-3.79 | 62.69 ($CH_2$) | 3.35 | 63.59 |
| **III** | **β-D-galactopyranoside 1** | | | |
| 1 | 4.49 | 104.72 (CH) | | |
| 2 | 3.19-3.79 | 75.86 (CH) | | |
| 3 | 3.19-3.79 | 80.27 (CH) | | |
| 4 | 3.19-3.79 | 71.69 (CH) | | |
| 5 | 3.19-3.79 | 77.90 (CH) | | |
| 6 | 3.19-3.79 | 62.80 ($CH_2$) | | |
| **IV** | **β-D-galactopyranoside 2** | | | |
| 1 | 4.51 | 104.93 (CH) | | |
| 2 | 3.19-3.79 | 76.00 (CH) | | |
| 3 | 3.19-3.79 | 81.02 (CH) | | |
| 4 | 3.19-3.79 | 72.45 (CH) | | |
| 5 | 3.19-3.79 | 78.50 (CH) | | |
| 6 | 3.19-3.79 | 63.09 ($CH_2$) | | |
| **V** | **Xylopyranoside** | | | |
| 1 | 4.85 | 104.57 (CH) | | |
| 2 | 3.19-3.79 | 73.18 (CH) | | |
| 3 | 3.19-3.79 | 75.16 (CH) | | |
| 4 | 3.19-3.79 | 70.40 (CH) | | |
| 5 | 3.19-3.79 | 67.19 ($CH_2$) | | |

NMR, Nuclear magnetic resonance.

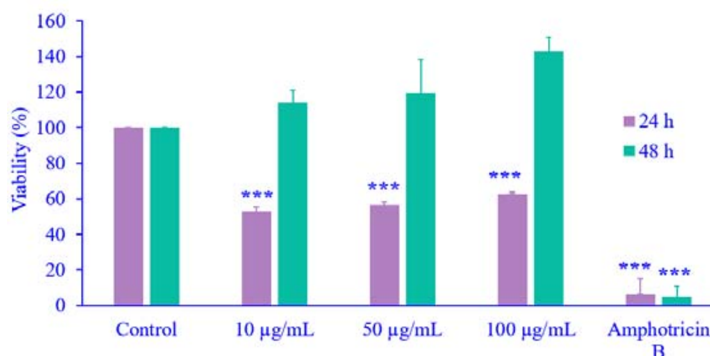


**Fig. 2.** Antileishmanial activities of different concentrations of compound (**1**). Data are expressed as mean ± SD, n = 3. ***$P \leq 0.001$ indicates significant differences in comparison with the corresponding control group.

***Antileishmanial activity of compounds (1) and (2)***

The antileishmanial activity of compounds (1) and (2) was assessed against the *L. major* promastigotes using the microplate method. The leishmanicidal activity of the compound (1) was significant in 10, 50, and 100 µg/mL after 24 h as shown in Fig. 2, but after 48 h its leishmanicidal activity decreased. Compound (2) showed a leishmanicidal effect after 24 h only in 10 µg/mL, while in 50 and 100 µg/mL and after 48 h the leishmanicidal activity was not significant (Fig. 3).

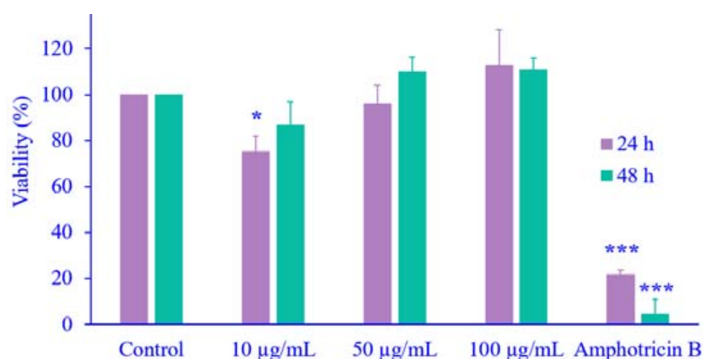


**Fig. 3.** Antileishmanial activities of different concentrations of compound **2.** Data are expressed as mean ± SD, n = 3. ***$P \leq 0.001$ indicates significant differences in comparison with the corresponding control group.

## DISCUSSION

As a member of the Amarillidaceae family, *A. giganteum*, an edible *Allium* species in Khorasan province of Iran, has been shown to possess a variety of pharmacological effects including antioxidant activity, inhibition of cAMP phosphodiesterase activity, *etc.* (17,18).

The phytochemical study of *A. giganteum*, especially the saponin constituents of the plant, resulted in the isolation and identification of two steroidal saponins from the flowers of the plant. Considering previous reports on the antimicrobial and especially antileishmanial activity of some natural steroidal saponins, the leishmanicidal effects of the isolated compound were evaluated, which exhibited its weak leishmanicidal activity on promastigotes of *L. major*. However, the activity has decreased significantly after 48 h which could be the result of metabolization, degradation, or bidesmosidic nature of isolated compounds. The results are in line with few recent reports about the antileishmanial activity of some steroidal saponins such as racemoside A isolated from *Asparagus racemosus* and the steroidal saponin isolated from *A. paradoxum* (8), which could be used as a chemical basis for justification of antimicrobial effects of different *Allium* species and scientific support of future studies of leishmanicidal steroidal saponins. Steroidal saponins in nature are found as glycosides which have features like frothing in water, hemolytic effect, being toxic for fishes, and complex formation with cholestrin (11). There are numerous scientific reports about the various pharmacologic effects of saponins such as anti-tumor effect, anti-fungal effect, inhibitory effect on abnormal bleeding of the uterus, chronotropic effect, and protective effect on gastric ulcers (12). In addition, these compounds have a broad spectrum of biological activities like hemolytic, hypocholesterolemic, immune system modulating, anti-inflammatory, anti-ulcer, and leishmanicidal effects (13,14).

To the best of our knowledge, this is the first time that compound 1 which is a cholestan saponin, isolated from *Allium* species and dioscoreavilloside A, achieved from *Dioscorea villosa* is similar to it with differences in substitution of a hydroxyl group and one of the sugars (19).

Species of the genus *Dioscorea* (family Dioscoreaceae) are widely used as botanical dietary supplements. These plants are well known for containing steroidal saponins, mainly belonging to the spirostanol and furostanol classes, and these have been used as chemical marker compounds for the quality control of botanical products. Wild yam, the rhizomes and roots of *Dioscorea villosa* L., is an important source of diosgenin (20)

Compound 2 is a furostanol saponin and is similar to parivispinoside A, which is achieved from *Tribulus parvispinus*, with a difference in a hydroxyl group substitution (21).

*Tribulus parvispinus* Presl (Zygophyllaceae) is an annual prostrate herb that grows in the warm regions of Egypt, Iraq, Iran, and Pakistan (22). Tribulus species are rich in furostane- and spirostane-type steroidal saponins that have displayed a wide range of biological activities including cytotoxic (23-26), antiproliferative (27), and antimicrobial effects (28).

## CONCLUSION

Phytochemical investigation of *A. giganteum* led to the isolation of two steroidal saponins with weak leishmanicidal activity from the plant for the first time, which established a valuable basis for further studies about steroidal saponins. The results are also of great importance for the explanation of the biological and pharmacological effects of the plant.

### *Acknowledgments*

The content of this paper is extracted from the Pharm. D thesis submitted by F. Chaparian which was financially supported by the Vice-Chancellery of Research of Isfahan University of Medical Sciences, Isfahan, Iran through Grant No. 399191.

### *Conflict of interest statement*

The authors declared no conflict of interest in this study.

### *Authors' contribution*

All the authors contributed equally to this work. The finalized article was read and approved by all authors.

## REFERENCES

1. Evans WC. Trease and Evans' pharmacognosy E-book. 16th ed. Elsevier Health Sciences; 2009. pp. 40.
2. Chehri Z, Zolfaghari B, Sadeghi Dinani M. Isolation of cinnamic acid derivatives from the bulbs of *Allium tripedale*. Adv Biomed Res. 2018;7:60,1-5. DOI: 10.4103/abr.abr_34_17.
3. Hosseinzadeh Namin H, Saeidi Mehrvarz S, Zarre S, Fritsch R. Pollen morphology of selected species of *Allium* (Alliaceae) distributed in Iran. Nord J Bot. 2009;27(1):54-60. DOI: 10.1111/j.1756-1051.2009.00319.x.
4. Movafeghi A, Miryeganeh M. Scape anatomy of *Allium* sect. *Allium* (Alliaceae) in Iran. J Sci. 2009;35(1):1-5.
5. Ozturk M, Gucel S, Altay V, Altundag E. Alliums, an underutilized genetic resource in the east Mediterranean. Acta Hort. 2012;969:303-310. DOI: 10.17660/ActaHortic.2012.969.39.
6. Lanzotti V. Bioactive polar natural compounds from garlic and onions. Phytochem Rev. 2012;11(2-3):179-196. DOI: 10.1007/s11101-012-9247-3.
7. Sadeghi M, Safaeian L, Aghaye Ghazvini MR, Ramezani M. Evaluation of fibrinolytic and antioxidant effects of *Allium affine* hydroalcoholic extract. Res Pharm Sci. 2017;12(4):299-306. DOI: 10.4103/1735-5362.212047.
8. Rezaee F, Zolfaghari B, Sadeghi Dinani M. Isolation of dioscin-related steroidal saponin from the bulbs of *Allium paradoxum L.* with leishmanicidal activity. Res Pharm Sci. 2018;13(5):469-475. DOI: 10.4103/1735-5362.236875.
9. Cui M, Song F, Zhou Y, Liu Z, Liu S. Rapid identification of saponins in plant extracts by electrospray ionization multi-stage tandem mass spectrometry and liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2000;14(14):1280-1286. DOI: 10.1002/1097-0231(20000730)14:14<1280::AID-RCM26>3.0.CO;2-C.
10. Sadeghi M, Zolfaghari B, Senatore M, Lanzotti V. Spirostane, furostane and cholestane saponins from Persian leek with antifungal activity. Food Chem. 2013;141(2):1512-1521. DOI: 10.1016/j.foodchem.2013.04.009.
11. Sang S, Mao S, Lao A, Chen Z, Ho CT. New steroid saponins from the seeds of *Allium tuberosum L.* J Agric Food Chem. 2001;49(3):1475-1478. DOI: 10.1021/jf001062b.
12. Zhang T, Liu H, Liu XT, Xu DR, Chen XQ, Wang Q. Qualitative and quantitative analysis of steroidal saponins in crude extracts from *Paris polyphylla* var. yunnanensis and *P. polyphylla* var. chinensis by high performance liquid chromatography coupled with mass spectrometry. J Pharm Biomed Anal. 2010;51(1):114-124. DOI: 10.1016/j.jpba.2009.08.020.
13. Adão CR, da Silva BP, Tinoco LW, Parente JP. Haemolytic activity and immunological adjuvant effect of a new steroidal saponin from *Allium ampeloprasum* var. porrum. Chem Biodivers. 2012;9(1):58-67. DOI: 10.1002/cbdv.201100005.
14. Adão CR, da Silva BP, Parente JP. A new steroidal saponin with antiinflammatory and antiulcerogenic properties from the bulbs of *Allium ampeloprasum* var. porrum. Fitoterapia. 2011;82(8): 1175-1180. DOI: 10.1016/j.fitote.2011.08.003.
15. Fritsch RM. A preliminary review of *Allium* subg. *Melanocrommyum* in central Asia. Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung Gatersleben (IPK), Gatersleben; 2016. pp. 168.
16. Oskuee RK, Jaafari MR, Amani S, Ramezani M. Evaluation of leishmanicidal effect of *Euphorbia erythadenia* extract by *in vitro* leshmanicidal assay using promastigotes of *Leishmania major*. Asian Pac J Trop Biomed. 2014;4(2):S581-S583. DOI: 10.12980/APJTB.4.2014C1018.
17. Štajner D, Milić-Demarino N, Čanadanović-Brunet J, Štajner M, Popović BM. Screening for antioxidant properties of *Allium giganteum*. Fitoterapia. 2006;77(4):268-270. DOI: 10.1016/j.fitote.2006.03.015.

18. Mimaki Y, Nikaido T, Matsomuto K, Sashida Y, Ohmoto T. New steroidal saponins from the bulbs of *Allium giganteum* exhibiting potent inhibition of cAMP phosphodiesterase activity. Chem Pharm Bull. 1994;42(3):710-714.
DOI: 10.1248/cpb.42.710.
19. Avula B, Wang YH, Wang M, Ali Z, Smillie TJ, Zweigenbaum J, *et al*. Characterization of steroidal saponins from *Dioscorea villosa* and *D. cayenensis* using ultrahigh performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry. Planta Med. 2014;80(4):321-329.
DOI: 10.1055/s-0033-1360330.
20. Dong SH, Cai G, Napolitano JG, Nikolić D, Lankin DC, McAlpine JB, *et al.* Lipidated steroid saponins from *Dioscorea villosa* (wild yam). Fitoterapia. 2013;91:113-124.
DOI: 10.1016/j.fitote.2013.07.018.
21. Perrone A, Plaza A, Bloise E, Nigro P, Hamed AI, Belisario MA, *et al*. Cytotoxic furostanol saponins and a megastigmane glucoside from *Tribulus p arvispinus*. J Nat Prod. 2005;68(10):1549-1553.
DOI: 10.1021/np0502138.
22. Kadria A. Ahmed, Amaal H. Mohamed. A taxonomic study of the genus *Tribulus* L. in Egypt. Arab Univ J Agric Sci. 2005;13(2):197-206.
23. Hu K, Dong A, Yao X, Kobayashi H, Iwasaki S. Antineoplastic agents II: four furostanol glycosides from rhizomes of *Dioscorea collettii var. hypoglauca*. Planta Med. 1997;63(2):161-165.
DOI: 10.1055/s-2006-957636.
24. Pan WB, Chang FR, Wei LM, Wu YC. New flavans, spirostanol sapogenins, and a pregnane genin from *Tupistra chinensis* and their cytotoxicity. J Nat Prod. 2003;66(2):161-168.
DOI: 10.1021/np0203382.
25. González AG, Hernández JC, León F, Padrón JI, Estévez F, Quintana J, *et al*. Steroidal saponins from the bark of *Dracaena draco* and their cytotoxic activities. J Nat Prod. 2003;66(6):793-798.
DOI: 10.1021/np020517j.
26. Dong M, Feng XZ, Wang BX, Wu LG, Ikejima T. Two novel furostanol saponins from the rhizomes of *Dioscorea panthaica* Prain et Burkill and their cytotoxic activity. Tetrahedron. 2001;57(3):501-506.
DOI: 10.1016/S0040-4020(00)01024-3.
27. Tran QL, Tezuka Y, Banskota AH, Tran QK, Saiki I, Kadota S. New spirostanol steroids and steroidal saponins from roots and rhizomes of *Dracaena a ngustifolia* and their antiproliferative activity. J Nat Prod. 2001;64(9):1127-1132.
DOI: 10.1021/np0100385.
28. Iorizzi M, Lanzotti V, Ranalli G, De Marino S, Zollo F. Antimicrobial furostanol saponins from the seeds of *Capsicum annuum L.* var. acuminatum. J Agric Food Chem. 2002;50(15):4310-4316.
DOI: 10.1021/jf0116911.