Records of Natural Products

www.acgpubs.org
ORIGINAL ARTICLE

Phytochemical and antileismanial activity studies on the tubers of *Cyclamen rohlfsianum* Asch

Ali Mansour Abdul Hafid Abdelmula[1], Kaya Süer[2], Emrah Güler[3], Ahmet Özbilgin[4], İbrahim Çavuş[4], Leonie Kayser[5] and İhsan Çalış[6*]

[1]Near East University, Faculty of Medicine, Department of Microbiology and Clinical Microbiology, Lefkoşa, Turkish Republic of Northern Cyprus
[2]Near East University, Faculty of Medicine, Department of Clinical Microbiology and Infectious Ilnesses, Lefkoşa, Turkish Republic of Northern Cyprus
[3]European University of Lefke, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Lefke, Turkish Republic of Northern Cyprus
[4]Manisa Celal Bayar University, Faculty of Medicine, Department of Medical Parasitology, Manisa, Türkiye
[5]University of Regensburg, Faculty of Chemistry and Pharmacy, Pharmaceutical Biology Department, Universitätsstraße 31, 93053 Regensburg, Germany
[6]Near East University, Faculty of Pharmacy, Department of Pharmacognosy Turkish Republic of Northern Cyprus

Abstract: Antileishmanial activity guided studies on the methanolic extract of the tubers of *Cyclamen rohlfsianum* Asch. resulted in the isolation and characterization of six saponins. All saponins were established as tri-, tetra-, and pentaglycosidic derivatives of 13β-28-epoxy-oleanane-type triterpenic sapogenols. Two of them were found to be newly described saponins, rohlfsianosides A and B. Their structures were identified as 3-*O*-β-{[β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-13β,28-epoxy-16α-hydroxy-30-acetoxy-oleanane (rohlfsianoside A), and 3-*O*-β-{{[β-D-xylopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→4)]-[β-D-glucopyranosyl-(1→4)]}-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-protoprimulagenin A (rohlfsianoside B), respectively. Additionally, three known compounds, cyclamiretin A (3β,16α-dihydroxy-13β-28-epoxy-oleanan-30-al) glycosides; cyclaminorin, deglucocyclamin and cyclamen, and protoprimulagenin A (13β-28-epoxy-3β,16α-dihydroxy-oleanane) derivative, lysikoianoside I, were identified. The structure elucidation of the saponins was established by means of spectroscopic methods (1D and 2D NMR, HR-MS). All the saponins showed notable growth inhibitory activity against *Leishmania tropica*, with IC_{50} values ranging from 17.5 to 21.9/mL.

Keywords: *Cyclamen rohlfsianum*, 13β-28-epoxy-oleanan-type saponins, rohlfsianosides A and B, antileishmanial activity, *Leishmania tropica*

Cite this article as:
Abdelmula et al. Phytochemical and antileismanial activity studies on the tubers of *Cyclamen rohlfsianum* Asch. (2026). *Records of Natural Products*, 20(1):e25093646

Received: *05 September 2025*
Revised: *21 September 2025*
Accepted: *24 October 2025*
Published: *03 November 2025*

1 Introduction

Neglected tropical diseases (NTDs) are a group of diseases caused by a variety of pathogens (including viruses, bacteria, parasites, fungi, and toxins) with devastating health, social, and economic arabinopyranoconsequences. NTDs mostly affect poor communities (over 1 billion people) in tropical regions, although some have a much wider geographical distribution. The World Health Organization (WHO) defines NTDs as diseases prevalent in the poorest regions of the world, where access to healthcare, sanitation, and safe drinking water is inadequate. During the COVID-19 pandemic, there has been an increase in mortality from NTDs due to inadequate support for individuals, often in the most vulnerable populations (World Health Organization (WHO), 2025; da Silva et al., 2024).

Leishmaniasis is one of the top ten infectious diseases among NTDs. It is a vector-borne infectious disease caused by *Leishmania* protozoa and affects more than 12 million people worldwide with 0.9–1.6 million new cases each year (da Silva et al., 2024). The disease is transmitted by sand flies (*Phlebotomus* spp.), and more than twenty *Leishmania* species are reported to cause human leishmaniasis (Köse & Temoçin, 2020). The primary reservoir of the parasites in

*Corresponding Author: İhsan Çalış.
Email: ihsan.calis@neu.edu.tr

nature is dogs and canids. It is also known that mice, foxes, cats, and humans can be reservoirs for these parasites. The disease is more common in tropical and subtropical regions than in other regions, and leishmaniasis cases have been reported from 98 countries in Europe, Africa, Asia and the Americas. Leishmaniasis remains an important public health problem in the Mediterranean basin, the Middle East, India, and South America (Ruh & Taylan Özkan, 2019; Güler et al., 2021, 2025).

Currently, the most commonly used antileishmanial drugs are pentavalent antimonials (N-methyl glucamine antimonate and sodium stibogluconate/SAG), amphotericin B, the antibiotic pentamidine, and miltefosine (oral anticancer drug) (Brindha et al., 2021). In addition, controlled-release drug systems developed by nanoparticles are considered as high-potential tools to improve antileishmanial chemotherapy (Iqbal et al., 2022; Khalid et al., 2022; Dar et al., 2020). Although SAG and meglumine antimonate are the first-line drugs for the treatment of leishmaniasis, SAG resistance has been reported to have become epidemic in some regions of India (Güler et al., 2021). Most of the drugs used in the treatment of leishmaniasis have serious side effects, especially nephrotoxic, hepatotoxic, and teratogenic effects (Direkel et al., 2016). Therefore, treatment of the disease is limited, toxic, costly, and inaccessible to patients due to repeated parenteral administration. Thus, the development of safer and shorter-term therapies is critical (da Silva et al., 2024).

In many regions where antileishmanial drugs are not available commercially, medicinal plants are used for treatment. Research has shown that natural products derived from plants can lead to new and effective compounds for the treatment of leishmaniasis (Güler et al., 2021). Furthermore, extracts from numerous traditional medicinal plants have been proven to exhibit inhibitory and even lethal effects against various pathogens, including *Leishmania* parasites (Bahmani et al., 2015; Delgado-Altamirano et al., 2017; Çolak, 2025; Passero et al., 2021; Saberi et al., 2024). Acetogenins, alkaloids, coumarines, curcumins, essential oils, flavonoids, neolignans, triglycerides, ursan- and oleanan-type triterpenes, cycloartenol glycosides are among the natural products reported for their antileishmanial activities (Çalış et al., 2006; Guimarães et al., 2010; Oryan, 2015; Özipek et al., 2005).

In this study, *C. rohlfsianum* was studied for its antileishmanial activity. *C. rohlfsianum* is native to a small area of northeastern Libya, in the Jabal Al Akhdar Mountain near the coast of Cyrenaica between Benghazi and Derna. It grows under scrub and in open woodland, on terra rossa over limestone, rocks, and in cavities, at an altitude of approximately 450 m. The tubers of the plants are traditionally used locally in Libya to treat Leishmania bites on the skin. The plant species *C. rohlfsianum* is a member of the *Cyclamen* genus and is widespread in regions of North Africa and the Mediterranean. The plant's medicinal ingredients are traditionally extracted by boiling its roots or tubers in water. Fomentation is another traditional use of this preparation, which involves applying a warm compress soaked in the decoction topically to the skin for therapeutic effects (El-Darier & El-Mogaspi, 2009).

Cyclamen L. species are members of Primulaceae together with *Androsace* L., *Lysimachia* Tourn. Ex L., and *Primula* L. *Ardisia* Sw., *Maesa* Forssk. and *Myrsine* L. are the members of family Myrsiniaceae. The common feature of these plants is the triterpenoid saponins with 13β,28-epoxy-oleanane-type sapogenols (Foubert et al., 2008). The sapogenol skeleton undergoes hydroxylation at positions C-3, C-16, C-21, C-22, and C-28, leading to varying degrees of hydroxylation. Depending on the number of hydroxyl groups introduced, these are diols, triols, tetrols, or pentols. The methyl groups located at C-23, C-29, and C-30 can be found in oxidized forms, including CH_2OH, CHO, and COOH, corresponding to increasing levels of oxidation. It can be suggested that hydroxylation and oxidation patterns on the oleanane skeleton, together with the sugar chain glycosylated to C-3, critically influence the saponin's physicochemical properties and bioactivity.

Cyclamen species are mostly used as ornamental flowers in gardens or in pots. On the other hand, they are used as herbal medicine for various purposes, such as antioxidant, anti-inflammatory, and anti-cancer activities. Moreover, antibacterial, antifungal, and uterine contractive activities, as well as antifertility effects, have also been reported (Çalış et al., 1997a, 1997b).

The crude extract of the tubers of *C. rohlfsianum* (CR-Ex), along with the fractions of the crude extract obtained by Vacuum Liquid Chromatography (VLC) (CR-A–CR-K) and the saponins isolated from the fractions CR-G, CR-H, and CR-I (**1**–**6**) (Figure 1) exhibited growth inhibitory activity against *Leishmania tropica* with IC_{50} values ranging from 17.5 to 21.9 µg/mL. Five of the saponins (**2**–**6**) were tested against *Leishmania tropica* promastigotes.

2 Materials and Methods

2.1 General Experimental Procedures

For VLC, a vacuum pump, and a column (41 mm × 400 mm) packed with LiChroprep RP-18 (Merck, Darmstadt) were used for the fractionation process. Silica gel (0.063–200 µM, Merck), LiChroprep C-18 (0.063–200 µM, Merck), and Sephadex LH-20 (Supelco) were used as stationary phases throughout chromatographic studies. Silica gel plates (Silica Gel 60 F_{254}, Merck) were used for Thin Layer Chromatography (TLC). Optical rotations were measured on a Schmidt+Haensch Polartronic MHZ-8 polarimeter. NMR measurements in CD_3OD were performed on a Bruker DRX 500 spectrometer (Bruker, Rheinstetten, Germany) operating at 500 MHz for ^1H and 125 MHz for ^{13}C, respectively, using the XWIN NMR software package for data acquisition and processing. The Finnigan TSQ 7000 HR-ESI [Thermo Fisher Scientific (Bremen) GmbH, Germany] was used in both negative and positive modes. For lyophlization a CHRIST Alpha 1–4 LD (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode/Harz, Germany) Plus was used.

http://doi.org/10.25135/rnp.2509.3646
Rec. Nat. Prod. 2026, 20(1):e25093646

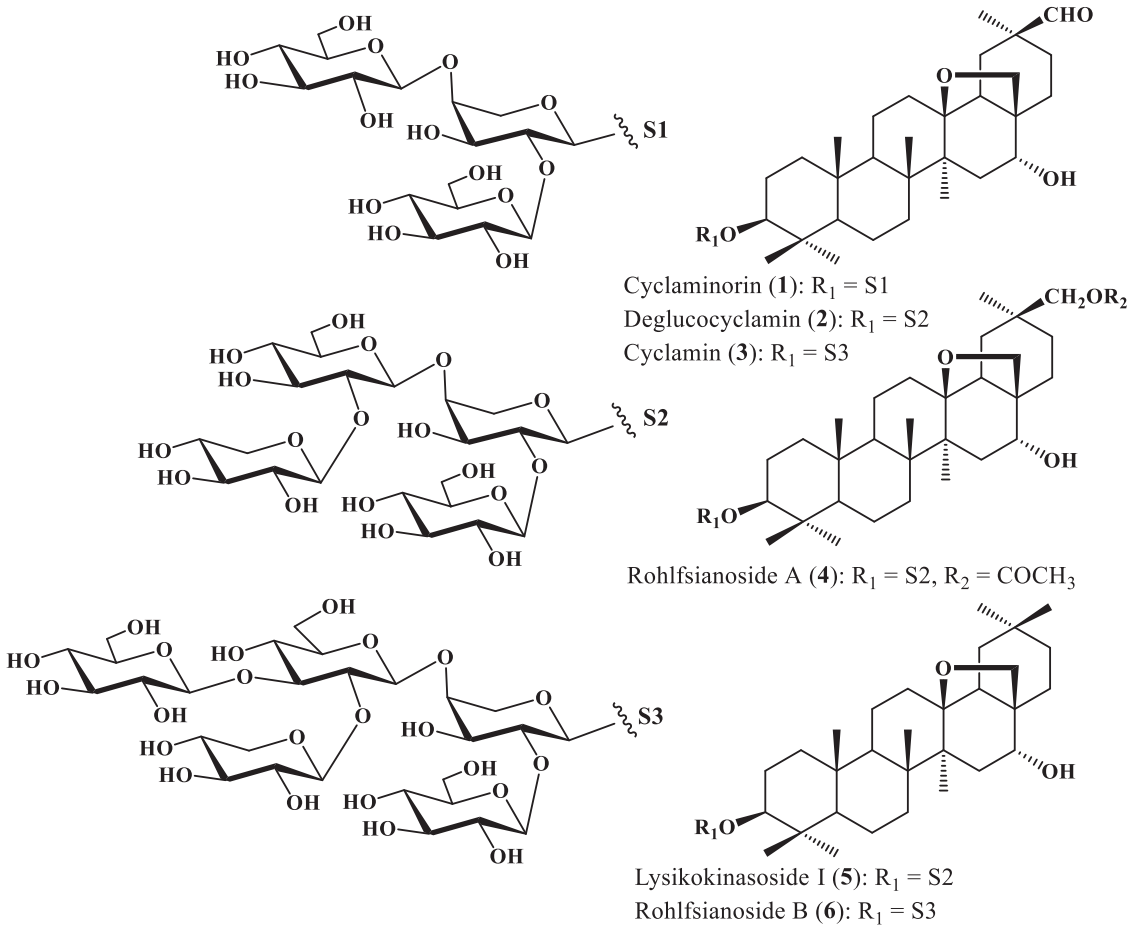

Figure 1. Saponins from the tubers of *C. rohlfsianum*

2.2 Plant Material

The plant material of *Cyclamen rohfsianum* (tubers) was collected from a region of N.E. Libya, Jabal Al-Akhdar Mountains (altitude 450 m) between Bengazi–Derna and identified by Prof. Dr. Mohamed Abuhadra and deposited as voucher specimen (6811) in the Herbarium of the Faculty of Science, Botany Department, the University of Tripoli in Libya.

2.3 Extraction and Fractionation

The air-dried, ground, underground parts (tubers, 200 g) of *C. rohfsianum* (CR) were extracted with 80% EtOH (2 × 800 mL) at 50°C using a rotary evaporator. The combined ethanolic extracts were evaporated under vacuum at 40°C, and the viscose residue was diluted with H_2O (~400 mL) and partitioned with n-BuOH three times (each 400 mL). *n*-BuOH phases were combined and evaporated until dryness, then dissolved in water (100 mL) and was lyophilized to give *n*-BuOH extract (CR-E: 23.287 g, yield 11.65%). A part of the n-BuOH extract (10 g) was applied to vacuum liquid chromatography (VLC) using LiChroprep-C18 as the stationary phase (100 g; ∅ 4.1 cm, h = 13.5 cm). For elution, H_2O and H_2O-MeOH mixtures with an increasing amount of MeOH in each 100 mL of the eluent. The first two fractions (frs. 1–2) were eluted only with H_2O (each 100 mL). The following thirteen fractions (frs. 3–15) were eluted using 10–95% MeOH in water (each 100 mL). Finally, only MeOH (100 mL, fr. 16) was used as an eluent. According to the results of TLC studies, fractions were combined into eleven fractions (CR A–K: frs. 2–3, 474 mg, A; frs. 4–6, 2158 mg, B; fr. 6, 880 mg, C; fr. 8, 322 mg, D; frs. 9–11, 843 mg, E; fr. 12, 532 mg, F; fr. 13, 2224 mg, G; fr. 14, 688 mg, H; fr. 15, 278 mg, J; fr. 16, 173 mg, K). Dichloromethan-MeOH-H_2O mixtures (80:20:2, 70:30:3 or 61:32:7) were used throughout the TLC studies.

According to the leishmanicidal activity results, frs. G, H and J were applied to silica gel column chromatography, separately, to afford saponin glycosides (**1**–**6**).

2.3.1. Isolation of Cyclaminorin (1), Deglucocyclamin (2) and Cyclamin (3)

A part of fr. G (1000 mg) was applied to a silica gel column (70 g) using a mixture of DCM-MeOH-H_2O with increasing polarity (80:20:1; 80:20:2; 75:25:2,5; 70:30:3; each 250 mL) as a solvent system (frs. volume: 13–14 mL). Fr. 35 yielded **1** (3 mg) while frs. 36–39 was a mixture of **1** and **2** (205 mg). Frs. 40–44 was pure in saponin **2** (248 mg). Frs. 45–50 was also rich in **2** (61 mg). Frs. 56–57 (20 mg) and 58–60 (64 mg) afforded compound **3**. The remaining fractions (frs. 61–57, 134 mg) were a mixture of compound **3** with a more polar saponin.

2.3.2. Isolation of Rohlfsianoside A (4)

Fr. H (688 mg) was applied to a silica gel column (70 g) using a mixture of DCM-MeOH-H_2O with increasing polarity (80:20:2; 70:30:3; 61:32:7; each 250 mL) as a solvent system. 50 fractions were collected (frs. volume: 13–14 mL). Frs. 1–32 (73 mg) and 33–36 (113 mg) yielded pure compound 4 (CR-H).

2.3.3. Isolation of Lysikoianoside (5) and Rohlfsianoside B (6)

Fr. J (260 mg) was applied to a silica gel column (25 g) using a mixture of $CHCl_3$-MeOH-H_2O with increasing polarity (90:10:1; 80:20:2; 70:30:3; 60:40:4; each 200 mL) as a solvent system. 63 fractions were collected (frs. volume: 12 mL). Frs. 8–45 gave compound **5** (67 mg), while frs. 55–61 gave compound **6** (43 mg).

2.3.4 Cyclaminorin (1)

Colourless amorphous powder; (+)-HR-MS *m/z* 951.49239 $[M+Na]^+$ (calcd. for $C_{47}H_{76}NaO_{18}$, Mol. Wt. 951.4929). Identified by co-chromatography with reference compound, cyclaminorin isolated from *Cyclamen coum* var. *coum* (Çaliş et al., 1997b).

2.3.5 Deglucocyclamin (2)

Colourless amorphous powder; (+)-HR-MS *m/z* 1083.53465 $[M+Na]^+$ (calcd. for $C_{52}H_{84}NaO_{22}$, Mol. Wt. 1083.5352). ^1H and ^{13}C-NMR (500 MHz and 125 MHz, respectively, CD_3OD) were in accordance with previously reported data from *Cyclamen coum* var. *coum* (Çaliş et al., 1997b).

2.3.6 Cyclamin (3)

Colourless amorphous powder; (+)-HR-MS *m/z* 1245.58747 $[M+Na]^+$ (calcd. for $C_{58}H_{94}NaO_{27}$, Mol. Wt. 1245.5880). ^1H and ^{13}C-NMR (500 MHz and 125 MHz, respectively, CD_3OD) were in accordance with those reported from *Cyclamen coum* var. *coum* (Çaliş et al., 1997b; Reznicek et al., 1989).

2.3.7 Rohlfsianoside A (4)

Colourless amorphous powder; $[\alpha]_D^{20}$ +50° (*c* 0.12, MeOH); (+)-HR-MS *m/z* 1127.56086 $[M+Na]^+$; (calcd. for $C_{58}H_{88}NaO_{23}$, Mol. Wt. 1127.5614). ^1H and ^{13}C-NMR (500 MHz and 125 MHz, respectively, CD_3OD) Tables 2A and 2B.

2.3.8 Lysikokinasoside I (5)

Colourless amorphous powder; (+)-HR-MS *m/z* 1069.55538 $[M+Na]^+$ (calcd. For $C_{52}H_{86}O_{21}Na$, Mol. Wt. 1069.5559). ^1H and ^{13}C-NMR (500 MHz and 125 MHz, respectively, CD_3OD) were in accordance with those reported from *Lysimachia sikokiana* and *L. candida* (Kohda et al., 1989; Zhang et al., 1999).

2.3.9 Rohlfsianoside B (6)

Colourless amorphous powder; $[\alpha]_D^{20}$ –140° (*c* 0.1, MeOH); (+)-HR-MS *m/z* 1231.60820 $[M+Na]^+$ (calcd. for $C_{54}H_{88}NaO_{23}$, Mol. Wt. 1231.6088). ^1H and ^{13}C-NMR (500 MHz and 125 MHz, respectively, CD_3OD) *see* Tables 3A and 3B.

2.4 Leishmania Tropica Strain

In our study, *Leishmania tropica* strain was used to investigate *in vitro* anti-leishmanial activity. The strain was MHOM/TR/2012/CBCL-LT coded strain stored in a liquid nitrogen tank nitrogen tank in the Parasite Bank of Manisa Celal Bayar University, Faculty of Medicine, Türkiye.

2.4.1 Culture of Leishmania tropica

Leishmania tropica promastigotes removed from liquid nitrogen were thawed in a 37°C water bath for two minutes. After thawing, they were inoculated into enriched NNN media and the media were incubated in an incubator at 26°C. The growth status of the promastigotes was checked every other day by preparing a slide-to-cover slip. The grown promastigotes were transferred from NNN medium to RPMI-1640 medium (GibcoTM, Paisley, Scotland, UK) containing 10% fetal calf serum (FCS), 1% penicillin/streptomycin, and 1% gentamicin and brought to the level of 107 promastigote/mL required for the study (Güler et al., 2021; Eser & Çavuş, 2023).

2.4.2 In Vitro Anti-leishmanial Study

In our study, anti-leishmanial activities of 23 different cyclamen derivatives (CC-3, CC-4, CC-5, CM-1, CM-3, CM-4, CM-6, CR-Ex, CR-A, CR-B, CR-C, CR-D, CR-E, CR-F, CR-G, CR-H, CR-I, CR-3, CR-G3, CR-G2, CR-H1, CR-J1, and CR-J2) were investigated. The 96-well cell culture plates (flat bottom) were used horizontally. Firstly, 100 µL of RPMI-1640 medium (containing 10% FCS, 1% penicillin/streptomycin, and 1% gentamicin) was transferred to all wells in triplicate. A different line on the plate was used as a drug control, and 100 µL Amphotericin B (AmpB) (0.06 µM) was added to the first well, and serial dilution was performed again. Then, 100 µL of *Leishmania tropica* promastigotes (concentration of 10^7 promostigotes/mL) was added to each well. In the negative control line, only 100 μL of promastigote suspension was placed, no active substance was added. After the procedures were completed, the plates were covered with parafilm and incubated in a 25°C incubator for 48 hours. XTT (Biotium, Fremont, CA, USA) (sodium 3,39-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulphonic acid monohydrate) cell viability test kit was prepared to measure absorbance, and, 50 µL of this solution was added to each well in the plate after incubation. The plates were incubated again at 37°C for 4 hours, and, then the IC_{50} values were determined by reading the absorbance at 450 nM. Viability percentages (%) for promastigotes were calculated according to the equation described in the previous study (Güler et al., 2021, 2025; Eser & Çavuş, 2023).

http://doi.org/10.25135/rnp.2509.3646
Rec. Nat. Prod. 2026, 20(1):e25093646

Table 1. Anti-leishmanial activity (IC_{50} in µg/mL) of the crude *n*-BuOH extract (CR-Ex), fractions of the crude extracts CR-Ex (CR-A–J) and the saponins **2–6**

Code of the samples	Crude extract, Fractions saponins (2–6)	IC_{50} µg/mL	IC_{50} mM/mL
CR-Ex (100 mg)	Crude extract (n-BuOH)	**24.41**	
CR-A (27 mg)	Fraction A	210.9	
CR-B (34 mg)	Fraction B	132.5	
CR-C (24 mg)	Fraction C	187.5	
CR-D (26 mg)	Fraction D	406.3	
CR-E (21 mg)	Fraction E	82.03	
CR-F (23 mg)	Fraction F	44.92	
CR-G (29 mg)	Fraction G	**14.16**	
CR-G2 (5.4 mg)	**Deglucoyclamin (2)**	**21.09**	**1.99**
CR-G3 (5.6 mg)	**Cyclamin (3)**	**21.88**	**1.79**
CR-H (19 mg)	Fraction H	**18.56**	
CR-H1 (5.3 mg)	**Rohlfsianoside A (4)**	**20.70**	**1.87**
CR-J (16 mg)	Fraction J	**15.63**	
CR-J1 (4.5 mg)	**Lysikoianoside I (5)**	**17.58**	**1.68**
CR-J2 (4.9 mg)	**Rohlfsianoside B (6)**	**19.14**	**1.58**
	AmpB Concentration (µM)		
AmpB*		0.063	
NC*		–	

Note: *) Abbreviations: AmpB = Amphotericin B; NC = Negative Control.

3 Results and Discussion

3.1 Antileishmanial Activity

The crude extract of the tubers of *C. rohlfsianum* (CR-Ex), and the fractions of the crude extract obtained by VLC (CR-A–CR-K), as well as the saponins (**2–6**) isolated from the fractions CR-G, CR-H, and CR-I (2–6), were evaluated in parallel for their antileishmanial activity (Table 1). Amphotericin B (AmpB) was used as the reference drug against *Leishmania tropica* promastigotes. The reference drug AmpB inhibited 50% of the promastigotes at a concentration of 0.063 µM in the fifth dilution. CR-Ex, CR-G, CR-H, CR-J were found to be the most effective against promastigotes (IC_{50}: 24.41 µg/mL, 14.16 µg/mL, 18.56 µg/mL, 15.63 µg/mL, respectively). All wells in the Negative Control (NC) line showed 100% promastigote viability. Isolation studies on the active fractions resulted in the isolation of the compounds **1–3** from CR-G, compound **4** from CR-H, and compounds **5** and **6** from CR-J. Saponin **1** was not studied due to the low yield. Saponins **2–6** exhibited growth-inhibitory activity against *Leishmania tropica*, with IC_{50} values ranging from 17.5 to 21.9 µg/mL (Table 1).

3.2 Structure Elucidation

Compound **1** was obtained as an amorphous powder. The co-chromatography of compound **1** together with the reference compound of cyclaminorin, isolated in a previous study, helped identify compound **1** as the same saponin (Çaliş et al., 1997b).

Compounds **2** and **3** were obtained as white, amorphous colourless powders. Based on their HR-MS and ^{1}H and ^{13}C-NMR data, their structures were established as tetra- and pentaglycosidic derivatives of cyclamiretin A (13β-28-epoxy-3,16-dihydroxy-olean-30-al), deglucocyclamin and cyclamin, respectively (Çalış et al., 1997a, 1997b; Reznicek et al., 1989).

Compound **4** was also obtained as an amorphous colourless powder. Its molecular formula was deduced as $C_{54}H_{88}O_{23}$ by HR-MS showing a sodium adduct ion peak at m/z 1127.56086 $[M+Na]^+$ (calcd. for $C_{54}H_{88}NaO_{23}$, Mol. Wt. 1127.5614). The ^{1}H-NMR spectrum of **4** was consistent for the presence of a 13β-28-epoxy-oleanan-type triterpene saponin with the existence of signal system observed as an AB system at δ 3.51 and 3.15 (J_{AB} = 7.3 Hz) and the carbon resonance at δ 78.68 (H_2-28 and C-28). The proton signals observed at δ 1.06, 0.85, 0.90, 1.15, 1.25 and 0.98 (each 3H, s) together with the corresponding carbon resonances at δ 28.52, 16.85, 16.87, 18.88, 20.14, and 28.86, respectively (Table 2A). By the help of HSQC and HMBC experiments, these methyl resonances were assigned to Me-23, Me-24, Me-25, Me-26, Me-27 and Me-29, respectively. Additionally, the ^{1}H,^{13}C-long-range correlations from these methyl resonances to the carbons of the oleanane skeleton allowed the assignment of all carbon and proton resonances of the oleanane skeleton (Table 2A). These findings led us to assign the two remaining methyl groups of the eight total methyl groups of the oleanane skeleton as a C-28 oxymethylene function and a hydroxymethylene at C-30. The latter was supported by the proton resonances observed as a second AB system at δ 4.26 and 3.83 (J_{AB} = 11.2 Hz) and the corresponding carbon resonances at δ 69.09 (C-30). In addition to these two oxygen-bearing methylene functionalities, two geminal protons on the oxygenated carbon atoms were observed at δ 3.15 and 3.94 and attributed to the H-3 and H-16, respectively. In the HSQC experiment,

Table 2A. The ^{1}H and ^{13}C-NMR data for the aglycone of rohlfsianoside A, **4** (δ_H 500 MHz; δ_C 125 MHz, CD_3OD, ppm)

	Rohlfsianoside A (4)			
C/H	δ_C	**DEPT**	δ_C, ***J*** **(Hz)**	**HMBC**
1	40.37	CH_2	1.78†/0.98†	
2	27.38	CH_2	1.87†/1.77†	
3	91.52	CH	3.15†	
4	40.76	C	–	
5	56.98	CH	0.72 d (10.2)	
6	18.88	CH_2	1.53†	
7	35.28	CH_2	1.56†/1.24†	
8	43.49	C	–	
9	51.77	CH	1.46†	
10	37.98	C	–	
11	20.14	CH_2	1.63†/1.48†	
12	33.36	CH_2	2.12†/1.35†	
13	88.45	C	–	
14	45.41	C	–	
15	37.19	CH_2	2.11†/1.24†	
16	78.22	CH	3.94†	
17	45.28	C	–	
18	51.51	CH	1.26	
19	34.34	CH_2	1.99†/1.29†	
20	36.13	C	–	
21	33.32	CH_2	2.12†/1.38†	
22	32.01	CH_2	1.81†/1.51†	
23	28.52	CH_3	1.06 s	C-3, C-5, C-24
24	16.85	CH_3	0.85 s	C-3, C-5, C-23
25	16.87	CH_3	0.90 s	C-1, C-5, C-8, C-9
26	18.88	CH_3	1.15 s	C-7, C-8
27	20.14	CH_3	1.25 s	C-8, C-13, C-14, C-15
28	78.68	CH_2	3.51 d/3.15 d J_{AB} = 7.3	
29	28.86	CH_3	0.98 s	C-19, C-20, C-21, C-30
30	69.09	CH_2	4.26 d/3.83 d J_{AB} = 11.2	C-29
C=O	173.13	C	–	C-30, CO ***CH***$_3$
CO ***C***H_3	20.89	CH_3	2.07 s	

the ^{1}H,^{13}C short-range correlations with these protons established the C-3 and C-16 resonances at δ 91.52 and 78.22, respectively. These findings strongly supported the presence of a 13β-28-epoxy-3,16,30-trihydroxy-oleanane skeleton as aglycone. The ^{1}H and ^{13}C-NMR resonances attributed to the sapogenol moiety were in good agreement with those of the sapogenol moiety of ardisicrenosides A, B (Jia et al., 1994), ardisianosides E–G (Chang et al., 2007) and ardisiacrispin E (Yin et al., 2022). Significant differences were observed in the chemical shifts of H_2-30 (δ 4.26 and 3.83 d, J_{AB} = 11.2 Hz) and C-30 (δ 69.9). The presence of an acetyl group was supported by the signal at δ 2.07 (3H, s) and carbonyl resonance δ 173.13. Thus, the downfield shift for the H_2-30 and C-30 resonances was predictive for the site of esterification, which was confirmed by the long-range correlations from H_2-30 and acetyl signals to the carbonyl resonance at δ 173.13 in the HMBC experiment. Based on these observations, the structure of the sapogenol moiety of compound **4** was established as 3β,16α-dihydroxy-30-acetoxy-13β-28-epoxy-oleanane. Four anomeric protons were observed at δ 4.40 (d, J = 6.4 Hz, H-1′), 4.69 (d, J = 7.7 Hz, H-1″), 4.51 (d, J = 7.6 Hz, H-1‴) and 4.50 (d, J = 7.8 Hz, H-1⁗). The proton signals in the same spin systems of each anomeric protons were established by COSY experiments, and corresponding carbon resonances were identified with the help of HSQC experiment. Finally, interglycosidic linkages between the sugar units and sapogenol moiety were consistent with the presence of a similar tetraglycosidic oligoholoside moiety consisting of an α-L-arabinose (δ 4.40, d, J = 6.4 Hz, H-1′), β-D-xylose (δ 4.50, d, J = 7.8 Hz, H-1⁗), and two β-D-glucose (δ 4.69, d, J = 7.7 Hz, H-1″; 4.51, d, J = 7.6 Hz, H-1‴) units similar to the those of deglucocyclamin. Based on these results, the structure of the compound **4** was determined as 3-*O*-β-{[β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-13β,28-epoxy-16α-hydroxy-30-acetoxy-oleanane. This is a

http://doi.org/10.25135/rnp.2509.3646
Rec. Nat. Prod. 2026, 20(1):e25093646

Table 2B. The ^{1}H and ^{13}C-NMR data of the sugar moiety of rohlfsianoside A, **4** (^{1}H-NMR: 500 MHz; ^{13}C-NMR: 125 MHz, CD_3OD, ppm)

		Rohlfsianoside A (4)			
	C/H	δ_C	**DEPT**	δ_H **ppm, *J* (Hz)**	**HMBC**
Ara-C(3)-Agly					
	1′	104.52	CH	4.40 d (6.4)	C-3
	2′	79.56	CH	3.81†	
	3′	74.46	CH	3.82†	
	4′	80.35	CH	3.90†	
	5′	65.87	CH_2	4.22 dd (12.6/3.2)/3.55†	C-1′
Glc_1-C(2′)-Ara					
	1″	105.74	CH	4.69 d (7.7)	C-2′
	2″	76.18	CH	3.21†	
	3″	78.21	CH	3.30†	
	4″	72.14	CH	3.19†	
	5″	78.06	CH	3.93†	
	6″	63.45	CH_2	3.86 dd/3.60	
Glc_2-C(3′)-Ara					
	1‴	104.95	CH	4.51 d (7.6)	C-4′
	2‴	85.29	CH	3.42	
	3‴	77.79	CH	3.40	
	4‴	71.26	CH	3.37	
	5‴	77.99	CH	3.31	
	6‴	62.72	CH_2	3.86 † 3.70 dd (12.0/6.6)	
Xyl-(C-2‴)-Glc_2					
	1⁗	107.48	CH	4.50 d (7.8)	C-2‴
	2⁗	76.06	CH	3.29	
	3⁗	77.74	CH	3.40	
	4⁗	71.12	CH	3.55	
	5⁗	67.57	CH_2	3.99 dd (11.4/5.3) 3.34 †	C-1⁗

Note: Abbreviations: Agly, aglycone; Ara, arabinose; Glc, Glucose; Xyl, xylose.
† Signal pattern unclear due to overlapping.

newly described 13*β*,28-epoxy-oleanane-type saponin and was named rohlfsianoside A.

Compound **5** was obtained as a colourless amorphous compound. The HR-MS spectrum showed the sodium adduct ion peak at *m/z* 1069.55538 $[M+H]^+$, corresponding to the molecular formula of $C_{52}H_{86}NaO_{21}$ (Mol. wt. 1069.5559). Contrary to the NMR spectra of the saponins **1**–**4**, the ^{1}H-NMR spectrum of **5** exhibited seven tertiary methyl signals at *δ* 1.23, 1.16. 1.06, 0.95, 0.91, 0.906 and 0.85 (each 3H, s). The proton signals arising an AB system at *δ* 3.12 and 3.50 (J_{AB} = 7.5 Hz, H_2-28) and a corresponding methylene carbon at *δ* 78.44 (C-28) were consistent with the existence of a 13*β*,28-epoxy functionality in **5**. These findings together with other resonances arising from the sapogenol moiety indicated that the sapogenol moiety was protoprimulagenin A (Çalis et al., 1992; Mua et al., 2010). Moreover, the signal arising from the tetraglycosidic oligosaccharide moiety was in accordance with those of deglucocyclamin (**2**) (Çalış et al., 1997a), and as well as rohlfsianoside A (**4**). Based on these results, the structure of **5** was determined as 3-*O*-*β*-{[*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)]-[*β*-D-glucopyranosyl-(1→2)]-*α*-L-arabinopyranosyl}-protoprimulagenin A. This compound was first reported from another member of the family Primulaceae, *Lysimachia* species and named as lysikokinasoside I (Kohda et al., 1989; Zhang et al., 1999). This compound has also been isolated from *Cyclamen repandum* (Dall'Acqua et al., 2010).

Compound **6** was obtained as a colourless amorphous compound. The positive ion HR-MS showed the sodium adduct ion peak at *m/z* 1231.60820 $[M+Na]^+$, which indicated a molecular formula of $C_{58}H_{96}NaO_{26}$ (calcd. mol. wt., 1231.6088). The assignments of all proton and carbon resonances were based on 2D-NMR (COSY, HSQC, and HMBC). Seven tertiary methyl signals and the protons at δ_H 3.50 and 3.12 (AB system, J_{AB} = 7.7 Hz, H_2-28) and the carbon resonance δ_C 78.89 (C-28) supported the presence of a 13*β*,28-epoxy bridge (Table 3A) revealing protoprimulagenin A as the sapogenol as observed for saponin **5**. On the other hand, the ^{1}H- and ^{13}C-NMR spectra of **5** were predictive of the presence of sugar units: δ_H 4.39, d, J = 6.4 Hz; δ_C 105.61 (H-1′ and C-1′ of the sugar attached to the aglycone, *α*-L-arabinose); δ_H 4.69, d, J = 7.7 Hz; δ_C 104.42 (H-1″ and C-1″ of *β*-D-glucose); δ_H 4.53, d, J = 8.0 Hz; δ_C 105.05 (H-1‴ and C-1‴ of the inner *β*-D-glucose); δ_H 4.72, d, J = 7.9 Hz; δ_C 105.71 (H-1⁗ and C-1⁗ of the terminal *β*-D-xylose); δ_H 4.65, d, J = 8.0 Hz; δ_C 104.42 (H-1′′′′′ and C-1′′′′′ of

Table 3A. The ^{1}H and ^{13}C-NMR data for the aglycone of rohlfsianoside B (**6**) (^{1}H-NMR: 500 MHz; ^{13}C-NMR: 125 MHz, CD_3OD, ppm).

			Rohlfsianoside B (6)	
C/H Atom	**δ_C**	**DEPT**	**δ_C, *J* (Hz)**	**HMBC**
1	39.94	CH_2	2.38 br dd (14.0/12.0)/1.20 †	C-25
2	27.39	CH_2	1.86 br dd (12.9/4.4)	
3	91.51	CH	3.17 dd (9.0/8.0)	C-1′
4	40.77	C	–	
5	56.99	CH	0.72 dd (11.4/1.2)	
6	18.88	CH_2	1.50†	
7	35.31	CH_2	1.53†/1.22†	
8	43.42	C	–	
9	51.53	CH	1.26†	
10	37.99	C	–	
11	20.02	CH_2	1.62 dd (12.9/4.4)/1.24†	
12	33.46	CH_2	2.04†/1.28†	
13	88.52	C	–	
14	45.44	C	–	
15	37.51	CH_2	2.10/1.17	
16	78.22	CH	3.89†	C-18, C-17
17	45.53	C	–	
18	52.51	CH	1.51†	
19	40.39	CH_2	1.76†/0.97†	
20	32.55	C	–	
21	37.20	CH_2	1.21†	
22	32.31	CH_2	1.78†/1.52†	
23	28.51	CH_3	1.06 s	C-4, C-5, C-24
24	16.90	CH_3	0.85 s	C-4, C-5, C-23
25	16.86	CH_3	0.90 s	C-5, C-9, C-10
26	18.96	CH_3	1.16 s	C-7, C-8, C-9, C-14
27	20.06	CH_3	1.23 s	C-8, C-13, C-14
28	78.89	CH_2	3.50 d/3.12 d (J_{AB} = 7.7)	C-17, C-18
29	34.06	CH_3	0.95 s	C-19, C-20, C-21, C-30
30	25.09	CH_3	0.91 s	C-19, C-20, C-21, C-29

Note: † Signal patterns unclear due to overlapping.

Table 3B. The ^{1}H and ^{13}C-NMR data of the sugar moiety of rohlfsianoside B, **6** (^{1}H-NMR: 500 MHz; ^{13}C-NMR: 125 MHz, CD_3OD, ppm)

			Rohlfsianoside B (6)		
	C/H	**DEPT**	**δ_C**	**δ_C, *J* (Hz)**	**HMBC**
Ara-C(3)-Agly					
	1′	CH	105.61	4.39 d (6.4)	C-3
	2′	CH	79.50	3.78†	
	3′	CH	74.35	3.79†	
	4′	CH	80.52	3.88†	
	5′	CH_2	65.74	4.21 dd (12.6; 2.7)/3.52†	C-1′
Glc_1-C(2′)-Ara					
	1″	CH	104.42	4.69 d (7.7)	C-2′
	2″	CH	75.71	3.19†	
	3″	CH	78.16	3.40–3.25	

http://doi.org/10.25135/rnp.2509.3646
Rec. Nat. Prod. 2026, 20(1):e25093646

Table 3B. (Continued)

	Rohlfsianoside B (6)				
	C/H	**DEPT**	δ_C	δ_C, ***J*** **(Hz)**	**HMBC**
	4″	CH	71.20	3.51†	
	5″	CH	78.03	3.40–3.25	
	6″	CH_2	62.52	3.87†/3.65†	
Glc_2-C(3′)-Ara					
	1‴	CH	105.05	4.53 d (8.0)	C-4′
	2‴	CH	82.09	3.58†	
	3‴	CH	87.39	3.70†	
	4‴	CH	71.96	3.17†	
	5‴	CH	77.48	3.40–3.25	
	6‴	CH_2	63.30	3.86†/3.64†	
Xyl-(C-2‴)-Glc_2					
	1⁗	CH	105.71	4.72 d (7.9)	C-2‴
	2⁗	CH	76.08	3.18†	
	3⁗	CH	78.07	3.40–3.25	
	4⁗	CH	69.90	3.40†	
	5⁗	CH_2	67.18	3.98 dd (11.7; 5.4)/3.28†	C-1⁗
Glc_3-(C-3‴)-Glc_2					
	1⁗′	CH	104.42	4.65 d (8.0)	C-3‴
	2⁗′	CH	75.35	3.25†	
	3⁗′	CH	78.20	3.88†	
	4⁗′	CH	71.54	3.30†	
	5⁗′	CH	77.78	3.40–3.25	
	6⁗′	CH_2	62.52	3.87†/3.65†	

Note: Abbreviations: Agly, aglycone; Ara, arabinose; Glc, Glucose; Xyl, xylose.

the terminal *β*-D-glucose) (Table 3B). The chemical shifts of the anomeric carbons indicated the pyranose forms of all sugar units.

The chemical shifts attributed to the penta-glycosidic oligosaccharide moiety, as well as the site of inter-glycosidic linkages were found to be the same as those of cyclamin (**3**). Significant $^{1}H,^{13}C$ long-range correlations were observed from H-1′ (δ_H 4.69) of the arabinose to C-3 (δ_C 91.51) of the protoprimulagenin A, from the anomeric proton (H-1″, δ_H 4.53) of one of the two terminal glucose units to C-2′ (δ_C 79.50) of arabinose, from the anomeric proton of the inner glucose (H-1‴, δ_H 4.53) to the C-4′ (δ_C 79.50) of arabinose, from the anomeric proton of the terminal xylose unit (H-1⁗, δ_H 4.53) to the C-2‴ (δ_C 82.09) of the inner glucose, and finally from the anomeric proton (H-1⁗′, δ_H 4.65) of the second terminal glucose unit to the C-3⁗ (δ_C 87.39) of the inner glucose unit confirming the same glycosidation pattern to those of cyclamin (**3**). Based on this evidence the structure of the saponin **6** was established as 3-*O*-*β*-{{[*β*-D-xylopyranosyl-(1→2)]-[*β*-D-glucopyranosyl-(1→4)]-[*β*-D-gIucopyranosyl-(1→4)]}-[*β*-D-glucopyranosyl-(1→2)]-*α*-L-arabinopyranosyl}-protoprimulagenin A which was named rohlfsianoside B as a newly described saponin.

4 Conclusion

Bioactivity-guided studies have shown that the antileishmanial compounds in *Cyclamen rohlfsianum* tubers are triterpenic saponins (**1**–**6**). All the saponins have 13*β*,28-epoxy-oleanane-type sapogenols and tri- (**1**), tetra- (**2**, **4**, **5**), and pentaglycosidic (**3** and **6**) monodesmosidic structures. Saponins (**2**–**6**) tested for antileishmanial activity exhibited notable growth inhibitory activity against *Leishmania tropica*, with IC_{50} values ranging from 17.5 to 21.9 μg/mL. Our findings are consistent with the results obtained with previously studied 13*β*,28-epoxy-oleanane-type saponins (Maes et al., 2004a, 2004b; Foubert et al., 2009; Vermeersch et al., 2009). According to these studies, 13,28-epoxy-oleanane triterpene saponins demonstrate potent and selective antileishmanial activity. On the other hand, the mechanism of action of the saponins can be attributed to their dual amphiphilic properties. Since the use of *C. rohlfsianum* tubers locally, saponins may interact with the membrane sterols of the promastigotes. As is well known, sterols play a crucial role in maintaining the functional integrity of cell membranes. Thus, saponins may act non-specifically and damage parasite cell membranes. Their local applications help concentrate their effect on the infected site, as reported by local users, meaning that the local use of *C. rohlfsianum* tubers may cause minimal systemic toxicity. Additionally, a recent study performed on the aerial parts of the same plant reported the isolation and characterization of flavonoids (genistein, kaempferol, hesperetin, and 7,8,4′-trihydroxyflavone), and a triterpene, oleanolic acid (Elabbar et al., 2014).

Acknowledgement

The authors kindly thank Prof. Dr. Hakan Göker (Ankara University, Faculty of Pharmacy, Türkiye) and Ayman Salkini (Prince Sattam Bin Abdulaziz University, Saudi Arabia) for 1D and 2D NMR measurements, Prof. Dr. Ahmet C. Gören (Gebze Technical University, Faculty of Sciences, Gebze, Kocaeli, Türkiye) for HRMS measurements, and Assist. Prof. Dr. Kumsal Kocadal Kaymakamzade for her feedback on the English quality of the article.

Author Contributions

Conceptualization, AMAH Abdelmula, K. Süer, İ Çalış; writing original draft preparation, İ Çalış, AMAH Abdelmula, E Güler; isolation and structure elucidation, İ Çalış; literature search and writing, L Kayser; antileishmanial activity studies, K Süer, E Güler, A Özbilgin, İ Çavuş.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflict of Interest

The authors declare they have no conflict of interest.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products.

ORCID

Ali Mansour Abdul Hafid Adelmula: 0009-0004-2278-478X
Kaya Süer: 0000-0002-2565-3425
Emrah Güler: 0000-0002-1635-0051
Ahmet Özbilgin: 0000-0003-3613-8741
İbrahim Çavuş: 0000-0002-3860-0146
Leonie Kayser: 0009-0006-0995-4697
İhsan Çalış: 0000-0001-5489-3420

References

Bahmani, M., Saki, K., Ezatpour, B., Shahsavari, S., Eftekhari, Z., Jelodari, M., Rafieiankopaei, M. & Sepahvand, R. (2015). Leishmaniosis phytotherapy: review of plants used in Iranian traditional medicine on leishmaniasis. *Asian Pacific Journal of Tropical Biomedicine*, *5*(9), 695–701. DOI: 10.1016/j.apjtb.2015.05.018.

Brindha, J., Balamurali, M. & Chanda, K. (2021). An overview on the therapeutics of neglected infectious diseases-leishmaniasis and chagas diseases. *Frontiers in Chemistry*, *9*, 622286.

Çalis, I., Yürüker, A., Rüegger, H., Wright, A. D. & Sticher, O. (1992). Triterpene saponins from *Primula veris* subsp. *macrocalyx* and *Primula elatior* subsp. *Journal of Natural Products*, *55*(9), 1299–1306. DOI: 10.1021/np50087a019.

Çalış, İ., Koyunoğlu, S., Yeşilada, A., Brun, R., Rüedi, P. & Taşdemir, D. (2006). Antityripanosomal cycloartane Glycosides from *Astragalus baibutensis*. *Chemistry & Biodiversity*, *3*, 923–929. DOI: 10.1002/cbdv.200690094/pdf.

Çalış, İ, Satana, M. E., Yürüker, A., Kelicen, P., Demirdamar, R., Alaçam, R., Tanker, N., Rüegger, H. & Sticher, O. (1997a). Triterpene saponins from *Cyclamen mirabile* and their biological activities. *Journal of Natural Products*, *60*(3), 315–318. DOI: 10.1021/np960658j.

Çalış, İ., Yürüker, A., Tanker, N., Wright, A. D. & Sticher, O. (1997b). Triterpene saponins from *Cyclamen coum* var. *coum*. *Planta Medica*, *63*(02), 166–170. DOI: 10.1055/s-2006-957637.

Chang, X., Li., W., Jia, Z., Satou, T., Fushiya, S. & Koike, K. (2007). Biologically active triterpenoid saponins from *Ardisia japonica*. *Journal of Natural Products*, *70*(2), 179–187. DOI: 10.1021/np0604681.

Çolak, G. (2025). Phytochemicals: potential drug candidates for leishmaniasis. In: Khan, M. K. (Ed.), *Recent advances in phytochemical research* (pp. 167–169). London, UK: IntechOpen Limited. DOI: 10.5772/intechopen.1007961.

da Silva, F., Rizk, Y. S., das Neves, A. R., Lourenço, E. M. G., Ferreira, A. M. T., Monteiro, M. M., de Lima, D. P., Perdomo, R. T., Bonfá, I. S., Toffoli-Kadri, M. C., Duarte, A. P., Nunes, D. M., Martines, M. A. U., Piranda, E. M. & de Arruda, C. C. P. (2024). Antileishmanial activity, toxicity and mechanism of action of complexes of sodium usnate with lanthanide ions: Eu(III), Sm(III), Gd(III), Nd(III), La(III) and Tb(III). *International Journal of Molecular Sciences*, *25*, 413. DOI: 10.3390/ijms25010413.

Dall'Acqua, S., Castagliuolo, I., Brun, P., Ditadi, F., Palù, G. & Innocenti, G. (2010). Triterpene glycosides with in vitro an-inflammatory activity from *Cyclamen repandum* tubers. *Carbohydrate Research*, *345*(5), 709–714. DOI: 10.1016/j.carres.2009.12.028.

Dar, M. J., McElroy, C. A., Khan, M. I., Satoskar, A. R. & Khan, G. M. (2020). Development and evaluation of novel miltefosine-polyphenol co-loaded second generation nano-transfersomes for the topical treatment of cutaneous leishmaniasis. *Expert Opinion on Drug Delivery*, *17*(1), 97–110. DOI: 10.1080/17425247.2020.1700227.

Delgado-Altamirano, R., Monzote, L., Piñón-Tápanes, A., Vibrans, H., Rivero-Cruz, J. F., Ibarra-Alvarado, C. & Rojas-Molina, A. (2017). In vitro antileishmanial activity of Mexican medicinal plants. *Heliyon*, *3*(9), e00394. DOI: 10.1016/j.heliyon.2017.e00394.

Direkel, Ş., Karaman, Ü., Tezcan Ülker, S., Utku, S., Aslan, G., Uysal, M., Şahin, M. F., Delialioğlu, N., Ülger, M. & Emekdaş, G. (2016). Investigation of anti-leishmanial activity of the ten different hydrazone derivatives. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, *22*, 519–524.

El-Darier, S. M. & El-Mogaspi, F. M. (2009). Ethnobotany and relative importance of some endemic plant species at El-Jabal El-Akhdar Region (Libya). *World Journal of Agricultural Sciences*, *5*, 353–360.

Elabbar, F. A., Habela, A. M., Bozkeha, N. M. A. & Awina, T. M. (2014). Isolation and identification of some compounds from *Cyclamen rohlfsianum* (Primulaceae) from Libya. *Scientific Reviews and Chemical Communications*, *4*, 1–10.

Eser, M. & Çavuş, İ. (2023). In vitro and in silico evaluations of the antileishmanial activities of new benzimidazole-triazole derivatives. *Veterinary Sciences*, *10*(11), 648. DOI: 10.3390/vetsci10110648.

Foubert, K., Theunis, M., Apers, S., Vlietinck, A. J. & Pieters, L. (2008). Chemistry, distribution and biological activities of 13,28-epoxy-oleanane saponins from the plant families myrsinaceae and primulaceae. *Current Organic Chemistry*, *12*(8), 629–642. DOI: 10.2174/138527208784577376.

Foubert, K., Vermeersch, M., Theunis, M., Apers, S., Cos, P., Claeys, M., Puyvelde, L. V., Pieters, L. & Maes, L. (2009). LC-MS analysis of 13,28-epoxy-oleanane saponins in *Maesa* ssp. Extracts with antileishmanial activity. *Phytochemical Analysis*, *20*(2), 159–167. DOI: 10.1002/pca.1112.

Guimarães, L. R. C., Rodrigues, A. P. D., Marinho, P. S. B., Muller, A. H., Guilhon, G. M. S., Santos, L. S., do Nascimento, J. L. M. & Silva, E. O. (2010). Activity of the julocrotine, a glutarimide alkaloid from *Croton pullei* var. *glabrior*, on *Leishmania* (L.) *amazonensis*. *Parasitology Research*, *107*(5), 1075–1081. DOI: 10.1007/s00436-010-1973-0.

Güler, E., Özbilgin, A., Çavuş, İ., Baddal, B., Etikan, İ., Başer, K. H. C. & Şanlıdağ, T. (2021). *In vitro* anti-leishmanial activity of essential oils extracted from plants growing in Northern cyprus against *Leishmania tropica*. *Turkiye Parazitoloji Dergisi*, *45*, 101–107. DOI: 10.4274/tpd.galenos.2021.6888.

Güler, E., Özbilgin, A., Çavuş, İ., Özel, Y., Gündüz, C., Süer, K. & Şanlıdağ, T. (2025). Molecular characterization of cutaneous leishmaniasis and canine leishmaniasis strains isolated from Northern Cyprus and their clinical reflections in mice models. *Mikrobiyoloji Bülteni*, *59*, 203–216. DOI: 10.5578/mb.202502149.

Iqbal, K., Khalid, S., McElroy, C. A., Adnan, M., Khan, G. M. & Dar, M. J. (2022). Triple-combination therapy for cutaneous leishmaniasis using detergent-free, hyaluronate-coated elastic nanovesicles. *Nanomedicine*, *17*(20), 1429–1447. DOI: 10.2217/nnm-2022-0077.

Jia, Z., Koike, K., Ohmoto, T. & Ni, M. (1994). Triterpenoid saponins from *Ardisia crenata*. *Phytochemistry*, *137*(5), 1389–1396. DOI: 10.1016/s0031-9422(00)90418-7.

Khalid, S., Salman, A., Iqbal, K., Rehman, F., Ullah, I., Saoskar, A. R., Khan, G. M. & Dar, M. J. (2022). Surfactant free synthesis of cationic nano-vesicles: a safe triple drug loaded vehicle for the topical treatment of cutaneous leishmaniasis. *Nanomedicine: Nanotechnology, Biology and Medicine*, *40*(1), 102490. DOI: 10.1016/j.nano.2021.102490.

Kohda, H., Takeda, O. & Tanaka, S. (1989). Molluscicidal triterpenoidal saponin from *Lysimachia sikokiana*. *Chemical and Pharmaceutical Bulletin*, *37*(12), 3304–3305. DOI: 10.1248/cpb.37.3304.

Köse, H. & Temoçin, F. (2020). Evaluation of adult cases of visceral leishmaniasis reported in turkey by pool analysis method. *Klimik Dergisi*, *33*, 157–162.

Maes, L., Germonprez, N., Quirijnen, L., Van Puyvelde, L., Cos, P. & Vanden Berghe, D. (2004a). Comparative activities of the triterpene saponin maesabalide III and liposomal amphotericin B (Ambisome) against *Leishmania donovani* in hamsters. *Antimicrobial Agents and Chemotherapy*, *48*(6), 2056–2060. DOI: 10.1128/aac.48.6.2056-2060.2004.

Maes, L., Vanden Berghe, D., Germonprez, N., Qurijnen, L., Cos, P., De Kimpe, N. & Van Puyvelde, L. (2004b). In vitro and in vivo activities of a triterpenoid saponin extract (PX- 6518) from the plant *Maesa balansae* against visceral *Leishmania* species. *Antimicrobial Agents and Chemotherapy*, *48*(1), 130–136. DOI: 10.1128/aac.48.1.130-136.2004.

Mua, L. -H., Gong., Q. -Q., Zhao, H. -X. & Liu, P. (2010). Triterpenoid Saponins from *Ardisia gigantifolia*. *Chemical and Pharmaceutical Bulletin*, *58*(9), 1248–1251. DOI: 10.1248/cpb.58.1248.

Oryan, A. (2015). Plant-derived compounds in treatment of leishmaniasis. *IJVR (Iranian Journal of Veterinary Research)*, *16*, 1–19.

Özipek, M., Dönmez, A. A., Çalış, İ, Brun, R., Rüedi, P. & Tasdemir, D. (2005). Leishmanicidal cycloartane-type triterpene glycosides from *Astragalus oleifolius*. *Phytochemistry*, *66*(10), 1168–1173. DOI: 10.1016/j.phytochem.2005.04.019.

Passero, L. F. D., Brunelli, E. D. S., Sauini, T., Amorim Pavani, T. F., Jesus, J. A. & Rodrigues, E. (2021). The potential of traditional knowledge to develop effective medicines for the treatment of leishmaniasis. *Frontiers in Pharmacology*, *8*, 690432. DOI: 10.3389/fphar.2021.690432.

Reznicek, G., Jurenitsch, J., Robien, W. & Kubelka, W. (1989). Saponins in *Cyclamen* species: configuration of cyclamiretin C and structure of isocyclamin. *Phytochemistry*, *28*(3), 825–828. DOI: 10.1016/0031-9422(89)80123-2.

Ruh, E., Taylan Özkan, A. (2019). Leishmaniasis in Northern Cyprus. *European Journal of Therapeutics*, *25*, 1–5.

Saberi, R., Jamshidzad, Z., Karimi, E., Abdi, J., Naserifar, R. & Mirzaei, A. (2024). Anti-leishmanial activity of *Hypericum Scabrum* extract against *Leishmania major*. *AMB Express*, *14*(1), 136. DOI: 10.1186/s13568-024-01800-6.

Salih, S. & Abdulrraziq, A. (2021). A morphological survey study of *Cyclamen rohlfsianum* Aschers plant in AlJabal Al Akhdar region - Libya. *Bayan Journal*, *10*, 291–301.

Sharara, A., Badran, A., Hijazi, A., Albahri, G., Bechelany, M., Mesmar, J. E. & Baydoun, E. (2024). Comprehensive review of cyclamen: development, bioactive properties, and therapeutic applications. *Pharmaceuticals*, *17*(7), 848. DOI: 10.3390/ph17070848.

Vermeersch, M., Foubert, K., da Luz, R. I., Puyvelde, L. V., Pieters, L., Cos, P. & Maes, L. (2009). Selective antileishmanial activity of 13,28 epoxy-oleanane and related triterpene saponins from the plant families myrsinaceae, primulaceae, aceraceae and icacinaceae. *Phytotherapy Research*, *23*(10), 1404–1410. DOI: 10.1002/ptr.2788.

World Health Organization (WHO). (2025). Neglected tropical diseases. Access date: 06.06.2025. Available from: https://www.who.int/health-topics/neglected-tropical-diseases#tab=tab_1.

Yin, X., Hu, R., Zhou, Y., Zhu, W. & Zhou, Y. (2022). Cytotoxic 13,28 epoxy bridged oleanane-type triterpenoid saponins from the roots of *Ardisia crispa*. *Molecules*, *27*(3), 1061. DOI: 10.3390/molecules27031061.

Zhang, X. R., Peng, S.-L., Xiao, S. -C. & Ding, L. -S. (1999). Saponins from *Lysimachia candida*. *Acta Botanica Sinica*, *41*, 534–536.