In Vitro Antiprotozoal Activity of Triterpenoid Constituents of Kleinia odora Growing in Saudi Arabia

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Abstract: Two lupane and four ursane triterpenes, namely epilupeol (1), lupeol acetate (2), ursolic acid (3), brein (4), 3β 11α-dihydroxy urs-12-ene (5) and ursolic acid lactone (6) were isolated from aerial parts of Kleinia odora and identified. Compounds 1 and 3–6 were isolated for the first time from K. odora. The triterpene constituents were investigated for antiprotozoal potential against erythrocytic schizonts of Plasmodium falciparum, intracellular amastigotes of Leishmania infantum and Trypanosoma cruzi and free trypomastigotes of T. brucei. Cytotoxicity was determined against MRC-5 fibroblasts to assess selectivity. The ursane triterpenes were found to be active against more than one type of the tested parasites, with the exception of compound 6. This is also the first report on the occurrence of ursane type triterpenes in the genus Kleinia and their antiprotozoal potential against P. falciparum, L. infantum, T. cruzi, and T. brucei.

Keywords: Kleinia odora; triterpenes; antiplasmodial; antileishmanial; antitrypanosomal
1. Introduction

Protozoal infections are a worldwide health problem, particularly in developing countries [1–4], and approximately 14% of the World population are at risk of infection. Various studies have been conducted on leishmaniasis, malaria, Chagas and sleeping sickness which are considered major killing diseases [5]. The drugs currently in use mostly lack adequate efficacy, are toxic or show other liabilities, such as the need for parenteral application or high cost [6]. This reflects the need to continue searching for new and better antiprotozoal drugs. Natural products may offer good sources of leads for new drug design and discovery.

The genus Kleinia is a flowering plant comprising 40 species that are distributed in Somalia, the Middle-East, Madagascar and India [7,8]. Three species of this genus are commonly distributed in the Southern regions in Saudi Arabia: K. odora (Forssk) DC, K. deflersii (O.Schwartz) and K. pendula (Forssk) Sch. Bip. [9–11]. Several species of Kleinia are known to be rich sources of oxygenated sesquiterpenoids such as germacrane and oplopane abrotanifolon derivatives and lupane-type tri-terpenoids [12–14]. Triterpenes comprise one of the most interesting groups of natural products due to their high potential as pharmacological agents, including leishmanicidal, trypanocidal and antiplasmodial activity [15].

As part of our ongoing research on Saudi medicinal plant metabolites with antiprotozoal potential, triterpenoid constituents from K. odora were isolated, structurally analyzed and evaluated for antiprotozoal potential against the protozoan parasites Plasmodium falciparum, Leishmania infantum, Trypanosoma cruzi and T. brucei. To assess selectivity, cytotoxicity was determined on MRC-5 fibroblasts.

2. Results and Discussion

2.1. Phytochemistry

Compound 1 was isolated as a white powder. Its $^{13}$C-NMR and DEPT spectrum (Table 1) exhibited 30 carbons, including seven singlet methyl groups, eleven methylenes, six methines one of which is oxygenated, and six quaternary carbons, which taken together revealed the basic skeleton of a pentacyclic triterpene. NMR spectra include signals for isopropenyl group (olefinic quaternary carbons at $\delta_c$ 150.9, methylene carbon at $\delta_c$ 109.3, protons singlets at $\delta_H$ 4.69 and 4.57, and methyl singlet at $\delta_H$ 1.64) suggesting lupane type triterpene. The $^1$H-NMR spectrum (Table 1) showed one oxymethylene proton at C-3 as a broad singlet ($\delta_H$ 3.39). The proton signal at $\delta_H$ 2.38 (1H, td, 11.0, 10.0, 5.5 Hz) further revealed a typical H-19β lupane structure [16]. By comparing these NMR data with previously published data [17], compound 1 was characterized as epilupeol.

Compound 2 was also isolated as white crystals. The NMR data (Table 1) suggested a lupane skeleton. The NMR data were identical with those of epilupeol, except for the ring A signals. Compound 2 displayed a double doublet at $\delta_H$ 4.47 confirming the $\alpha$-orientation of the C-3 proton [18]. It also showed additional signals attributed to the presence of an acetoxyl group (carbonyl signal at $\delta_C$ 171.0 and methyl signals at $\delta_C$ 27.9 and $\delta_H$ 2.04). By comparison with previously reported data [19], compound 2 was identified as lupeol acetate.

Compound 3 was isolated as a white powder. The $^1$H- and $^{13}$C-NMR spectra (Table 1) revealed the presence of five tertiary methyls, two secondary methyls, nine methylenes, seven methines, one of
which was oxygenated, and six quaternary carbons, which indicated a pentacyclic triterpenoid. The NMR spectrum showed a double bond (δC 124.5, 138.2 ppm) and a carboxyl group (δC 178.6) signals. The positions of the carboxy group and double bond were confirmed by a HMBC correlation study. By comparison with previously reported data [20] compound 3 was identified as ursolic acid (3β-hydroxy-urs-12-en-28-oic acid).

The proton and carbon signals in the 1H- and 13C-NMR spectra (Table 1) of compound 4 were very similar to those of compound 3, except for the signals of ring D. The carboxy group signals in the NMR spectrum disappeared and it showed one additional oxymethine signal (δH 4.19; δC 67.4) and an additional methyl singlet at δH 0.80. The hydroxyl and methyl groups were positioned at C-16 and C-17 based on a HMBC correlation study. Comparing the spectral data with those reported in the literature [21], compound 4 was identified as brein (urs-12-ene-3β,16β-diol).

The 13C-NMR data of compound 5 (Table 1) were very similar to those of compound 4 and differed in the signals for rings C and D. This was attributed to the presence of an α-hydroxy at C-11 and the disappearance of the β-hydroxyl at position 16. Spectral data of 5 are reported for the first time based on DEPT, HMBC and HSQC evaluation and comparison of NMR data of structurally related compounds [21] and the published 1H-NMR data of its acetate form [22]. Compound 5 was identified as 3β, 11α-dihydroxy urs-12-ene.

The NMR spectral data of compound 6 (Table 1) exhibited characteristic signals of an oleanan-28,13β-olide. The 13C-NMR spectrum displayed resonances for a double bond at δC 129.9 and 135.0 for C-11 and C-12, in addition to an oxygenated quaternary carbon at 91.9 and a carbonyl at 182.6 for C-13 and C-28, respectively. These data confirmed the olean-11-en,28,13β-olide structure. These results are in agreement with previously reported [23] data for ursolic acid lactone 6. Compounds 1 and 3–6 were isolated for the first time from K. odora. To the best of our knowledge, this is also the first report on the occurrence of ursane type triterpenes in the genus Kleinia. The structures are summarized in Figure 1.

2.2. Antiprotozoal Activity

Petroleum ether and chloroform extracts of K. odora exhibited potent activity against T. brucei with IC50 values of 0.5 µg/mL (Table 2). The chloroform extract gave slightly higher selectivity (SI = 63) compared to the petroleum ether extract (SI = 39). The chloroform extract also displayed moderate activity against P. falciparum schizonts and intracellular amastigotes of L. infantum (IC50 8 µg/mL). In comparison, the petroleum ether extract showed similar activity against P. falciparum, L. infantum and T. cruzi (IC50 8.6, 6.8 and 5.7 µg/mL) but with lower selectivity. This motivated us to investigate the antiprotozoal activity of the isolated and identified triterpenoids from the chloroform extract.

Brein (4) and 3β, 11α-dihydroxy urs-12-ene (5) were found to be active and selective against more than one of the investigated protozoa. Brein (4) showed selective and potent activity against T. brucei (IC50 2.3 µM, SI > 27.8), with moderate side-activity against P. falciparum, L. infantum and T. cruzi (IC50 9.3–9.9 µM) and acceptable selectivity (SI > 6.5). Compound 5 showed potent and selective activity against L. infantum (IC50 3.2 µM, SI > 20) with some side-activity against T. cruzi and T. brucei (IC50 8.1 and 7.9 µM, respectively, SI > 7.9).
Figure 1. Structures of the triterpenoid compounds 1–6.

1. R₁ = H, R₂ = OH
2. R₁ = OCOCH₃, R₂ = H
3. R₁ = H, R₂ = COOH, R₃ = H
4. R₁ = OH, R₂ = CH₃, R₃ = H
5. R₁ = H, R₂ = CH₃, R₃ = OH
6.

Although ursolic acid (3) showed efficacy against *T. brucei* (IC₅₀ 2.2 µM) comparable to that of brein (4), its effect against *T. cruzi* and *L. infantum* (IC₅₀ 8.8 and 7.4 µM) was associated with low selectivity. Meanwhile, ursolic acid lactone 6 was devoid of any antiparasitic activity at the highest concentration tested (64 µg/mL). The antiparasomal and antileishmanial activity of ursolic acid obtained herein is in consistent with the data found in literature. It has been shown to be active against *T. brucei* at IC₅₀ 1.0 ± 0.2 µg/mL (2.2 µM) [24,25]. In addition, it was also claimed active against *T. cruzi* with IC₅₀ 21 µM [26] and *L. donovani* (IC₅₀ = 3.5 µg/mL) (7.6 µM) [27]. It is worthy of pointing out that in our investigation, the antiparasomal activity of this compound (IC₅₀ 29.7 µM) is not in agreement with previously reported data which lists IC₅₀ values of 3.1 µg/mL (6.8 µM) [28] and 4.9 µg/mL (10.7 µM) [29].
Table 1. $^1$H- and $^{13}$C-NMR data for compounds 1–6.

| № | Compound 1 in CDCl$_3$ | Compound 2 in CDCl$_3$ | Compound 3 in DMSO | Compound 4 in MeOD | Compound 5 in CDCl$_3$ | Compound 6 in MeOD |
|---|------------------------|------------------------|-------------------|-------------------|------------------------|-------------------|
| # | δ$_C$ | δ$_H$ | δ$_C$ | δ$_H$ | δ$_C$ | δ$_H$ | δ$_C$ | δ$_H$ | δ$_C$ | δ$_H$ | δ$_C$ | δ$_H$ |
| 1 | 33.2 | 38.4 | 38.2 | 40.1 | 39.7 | 39.4 |
| 2 | 25.1 | 21.3 | 28.2 | 27.9 | 27.4 | 27.7 |
| 3 | 76.3 | 3.39 (br s) | 81.0 | 4.47 (dd, 5.5, 10.5 Hz) | 76.8 | 79.7 | 3.18 (d, 7.5 Hz) | 78.8 | 3.23 (br s) | 79.5 | 3.2 (dd, 6.5, 10 Hz) |
| 4 | 37.5 | 38.0 | 38.8 | 39.9 | 39.1 | 40.0 |
| 5 | 49.0 | 54.8 | 54.8 | 56.7 | 55.3 | 56.1 |
| 6 | 18.3 | 18.0 | 19.5 | 18.4 | 18.8 |
| 7 | 34.1 | 32.7 | 31.8 | 33.5 | 32.3 |
| 8 | 41.0 | 40.8 | 41.0 | 43.3 | 41.5 |
| 9 | 50.1 | 47.1 | 48.5 | 55.8 | 2.2 (d, 10 Hz) | 54.0 |
| 10 | 37.2 | 36.7 | 36.8 | 38.1 | 37.5 |
| 11 | 20.8 | 22.9 | 24.2 | 68.3 | 4.26 | 129.9 | 5.61 (d, 8.5 Hz) |
| 12 | 25.3 | 124.5 | 5.16 (br s) | 126.3 | 5.23 (br s) | 128.6 | 5.18 (d, 2 Hz) | 135.0 | 6.07 (d, 10 Hz) |
| 13 | 38.0 | 37.1 | 138.2 | 139.7 | 143.0 | 91.9 |
| 14 | 42.9 | 41.6 | 45.1 | 42.1 | 42.0 |
| 15 | 27.4 | 25.1 | 36.4 | 27.9 | 26.6 |
| 16 | 35.6 | 25.6 | 67.4 | 4.19 (d, 6.5 Hz) | 27.4 | 23.9 |
| 17 | 43.0 | 46.7 | 39.5 | 33.6 | 46.6 |
| 18 | 48.3 | 52.4 | 2.11 | 62.4 | 58.0 | 61.8 |
| 19 | 48.0 | 2.38 (dd, 11, 10, 5.5 Hz) | 48.0 | 2.38 (dd, 11, 10, 5.5 Hz) | 38.4 | 40.9 | 39.3 | 41.5 |
| 20 | 150.9 | 151.0 | 38.1 | 40.0 | 39.4 | 39.4 |
| 21 | 29.8 | 30.4 | 31.8 | 31.1 | 32.3 |
| 22 | 40.0 | 36.5 | 36.8 | 41.3 | 32.4 |
| 23 | 28.3 | 27.4 | 0.85 (s) | 21.1 | 0.90 (s) | 28.8 | 0.82 (s) | 28.7 | 28.3 | 0.96 (s) |
| 24 | 22.2 | 16.5 | 0.84 (s) | 16.9 | 0.68 (s) | 16.3 | 1.02 (s) | 23.1 | 1.29 (s) | 15.6 | 0.80 (s) |
Table 1. Cont.

| #  | Compound 1 in CDCl₃ | Compound 2 in CDCl₃ | Compound 3 in DMSO | Compound 4 in MeOD | Compound 5 in CDCl₃ | Compound 6 in MeOD |
|----|---------------------|---------------------|--------------------|--------------------|---------------------|---------------------|
|    | δC                | δH                  | δC                | δH                  | δC                | δH                  |
| 25 | 16.0              | 0.84 (s)            | 16.2              | 1.05 (s)            | 16.4              | 1.02 (s)            |
| 26 | 15.9              | 1.03 (s)            | 16.0              | 0.83 (s)            | 16.9              | 0.76 (s)            |
| 27 | 14.6              | 0.96 (s)            | 14.5              | 0.79 (s)            | 23.3              | 1.10 (s)            |
| 28 | 18.0              | 0.78 (s)            | 18.0              | 0.94 (s)            | 178.6             | 22.1                |
| 29 | 109.3             | 4.69 (br s), 4.57 (br s) | 169.3             | 0.82, (d, 6.5 Hz)  | 17.0              | 0.84 (d, 6.5 Hz)    |
| 30 | 19.3              | 1.64                | 21.1              | 0.92, (d, 7.0 Hz)  | 21.1              | 0.97 (d)            |
| 1' |                   |                     | 171.0             |                     |                   |                     |
| 2' |                   |                     |                   |                     |                   |                     |
|    | 27.9              | 2.04 (s)            |

Table 2. Antiprotozoal activity and cytotoxicity of triterpenoid constituents isolated from the plant K. odora.

| Sample                   | P. falciparum | L. infantum | T. cruzi | T. brucei | MRC-5 |
|--------------------------|---------------|-------------|----------|-----------|-------|
|                          | IC₅₀ µg/mL    | SI          | IC₅₀ µg/mL | SI       | IC₅₀ µg/mL | SI   | IC₅₀ µg/mL |
| Petroleum ether extract  | 8.6 ± 2.1     | 2.3         | 6.8 ± 1.6 | 3        | 5.7 ± 1.6  | 3.4  | 0.5 ± 0.1  | 39       | 19.4 ± 3.4 |
| Chloroform extract       | 8.2 ± 1.9     | 4           | 8.1 ± 2.3 | 4        | 31.0 ± 4.9 | -   | 0.5 ± 0.1  | 63       | 31.3 ± 4.2 |
| Cmpd. 3                  | 29.7 ± 5.9    | <1          | 7.4 ± 1.9 | 1.5      | 8.8 ± 2.3  | 1.3  | 2.2 ± 0.6  | 5.2      | 11.4 ± 2.1 |
| Cmpd. 4                  | 9.7 ± 3.2     | >6.6        | 9.3 ± 2.2 | >6.9     | 9.9 ± 2.6  | >6.5 | 2.3 ± 0.4  | >27.8    | >64.0     |
| Cmpd. 5                  | 23.9 ± 5.7    | 2.7         | 3.2 ± 0.9 | >20      | 8.1 ± 1.8  | >7.9 | 7.8 ± 1.8  | >8.2     | >64.0     |
| Cmpd. 6                  | >64.0         | -           | >64.0     | -        | >64.0     | -   | 40.9 ± 8.1 | -        | >64.0     |
| Chloroquine              | 0.3 ± 0.05    | 10.4 ± 2.1  | 1.9 ± 0.3 | 0.03 ± 0.01 | 11.4 ± 3.2 |
| Miltefosine              |               | 0.3 ± 0.05  | 10.4 ± 2.1|          |         |
| Benznidazole             |               | 1.9 ± 0.3   |          |         |
| Suramine                 |               | 0.03 ± 0.01 |          |         |
| Tamoxifen                |               |             |          |         |

IC₅₀ µg/mL for extracts; µM for pure compounds.
These divergent results may be attributed to the diversity of parasite strains, parasite load, stages of parasite life cycle or be related to differences in the experimental conditions [30]. Ursolic acid lactone 6 was reported to have activity against L. donovani (IC$_{50}$ 191.52 µM) without cytotoxicity towards peripheral blood mononuclear cells [31]. In our opinion, this result was misinterpreted by the authors since the standard miltefosine under the same experimental conditions showed an IC$_{50}$ of 9.31 µM [31]; hence, the compound should be considered as inactive. To the best of our knowledge, this is the first report on the antiprotozoal evaluation of compounds 4, 5 and 6 against P. falciparum, L. infantum, T. cruzi, and T. brucei.

3. Experimental

3.1. General

The 1D-NMR and 2D-NMR spectra were recorded on a Bruker AMX-400 spectrometer with tetramethysilsilane (TMS) as an internal standard. Thin layer chromatography (TLC) was performed on precoated silicagel F254 plates (E. Merck, Darmstadt, Germany). All chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

3.2. Plant Materials

The plant Kleinia odora was collected from the South of Saudi Arabia in February 2011 and identified at the Pharmacognosy Department, College of Pharmacy, King Saud University. A voucher specimen was deposited at the Pharmacognosy Department, College of Pharmacy, King Saud University (Voucher # P-15129).

3.3. Extraction and Isolation

The air-dried and powdered aerial part of K. odora (1 kg) was extracted by maceration with 70% ethanol (4 × 2 L) at room temperature. The combined obtained ethanolic extract was filtered and evaporated at 40 °C using a rotary evaporator. The dried ethanolic extract (50 g) was subsequently redissolved in water (200 mL) and partitioned successively for several times with petroleum ether (3 × 200 mL), chloroform (3 × 200 mL) and n-butanol (3 × 200 mL) to provide the corresponding extracts. The petroleum ether extract (6 g) was subjected to column chromatography on pre-packed silica gel columns (35 mm i.d. × 350 mm) to give nine fractions. The elution was performed with a gradient of hexane-ethyl acetate (10:1) to pure ethyl acetate. TLC analysis of the fractions with anisaldehyde/sulfuric acid and heating at 100 °C allowed the analysis of the nine fractions. Fraction 1 was further purified by using a chromatotron (Harrison Research, Palo Alto, CA, USA) (silica gel 60 F$_{254}$, layer thickness 2 mm, Merck, Darmstadt, Germany) to give compound 2 (27 mg). The elution was performed with a mobile phase composed of hexane–dichloromethane (60:40, v/v). The chloroform extract (4 g) was applied on a silica gel column and eluted with a gradient of dichloromethane-ethyl acetate (9:1) to pure ethyl acetate to give seven fractions. Fraction 1 was rechromatographed on a silica gel column (dichloromethane-acetone, 9:1) and on a RP-18 column (MeOH–H$_2$O, 90:10) to produce compound 4 (48 mg) and compound 6 (21 mg). Direct crystallization of fraction 2 eluted by 30% acetone-dichloromethane gave compound 3 (35 mg). Fraction 5 was purified on a
RP-18 column (MeOH–H₂O, 90:10) to give compound 5 (8 mg). Fraction 7 (80 mg) was subjected to RP-18 column chromatography with MeOH–H₂O (90:10) as a solvent to produce compound 1 (20 mg).

**Epilupeol (1).** Amorphous powder; m.p. 205 °C; NMR (CDCl₃): see Table 1.

**Lupeol acetate (2).** White crystals; m.p. 215–218 °C; NMR (DMSO): see Table 1.

**Ursolic acid (3).** White powder; m.p. 236 °C; NMR (DMSO): see Table 1.

**Urs-12-ene-3β,16β-diol (4).** Solid; m.p. 221 °C; NMR (MeOD): see Table 1.

**3β,11α-Dihydroxyurs-12-ene (5).** Amorphous powder; NMR (CDCl₃): see Table 1.

**3-Hydroxy-13,28-epoxyurs-11-en-28-one (6, ursolic acid lactone).** White powder; m.p. 268 °C; NMR (MeOD): see Table 1.

### 3.4. Antiprotozoal Assay

#### 3.4.1. Standard Drugs

For the different tests, appropriate reference drugs were used as positive control: tamoxifen for MRC-5, chloroquine for *P. falciparum*, miltefosine for *L. infantum*, benznidazole for *T. cruzi* and suramin for *T. brucei*. All reference drugs were either obtained from the fine chemical supplier Sigma-Aldrich, Taufkirchen, Germany (tamoxifen, suramin) or from WHO-TDR, Geneva, Switzerland (chloroquine, miltefosine, benznidazole).

#### 3.4.2. Biological Assays

The integrated panel of microbial screens and standard screening methodologies were adopted as previously described [32]. All assays were performed in triplicate at the Laboratory of Microbiology, Parasitology and Hygiene at the University of Antwerp (Belgium). Plant extracts were tested at 5 concentrations (64, 16, 4, 1 and 0.25 μg/mL) to establish a full dose-titration and determination of the IC₅₀ (inhibitory concentration 50%). The final in-test concentration of DMSO did not exceed 0.5%, which is known not to interfere with the different assays [32]. Selectivity of activity was assessed by simultaneous evaluation of cytotoxicity on a fibroblast (MRC-5) cell line. The criterion for activity was an IC₅₀ < 10 μg/mL and a selectivity index (SI) of > 4.

#### 3.4.3. Antiplasmodial Activity

Chloroquine-resistant *P. falciparum* K 1-strain was cultured in human erythrocytes O⁺ at 37 °C under a low oxygen atmosphere (3% O₂, 4% CO₂, and 93% N₂) in RPMI-1640, supplemented with 10% human serum. Infected human red blood cells (200 μL, 1% parasitaemia, 2% haematocrit) were added to each well and incubated for 72 h. After incubation, test plates were frozen at −20 °C. Parasite multiplication was measured using the Malstat assay, a colorimetric method based on the reduction of 3-acetyl pyridine adenine dinucleotide (APAD) by parasite-specific lactate-dehydrogenase (pLDH) [32,33].
3.4.4. Antileishmanial Activity

*L. infantum* MHOM/MA(BE)/67 amastigotes were collected from the spleen of an infected donor hamster and used to infect primary peritoneal mouse macrophages. To determine *in vitro* antileishmanial activity, $3 \times 10^4$ macrophages were seeded in each well of a 96-well plate. After 2 days outgrowth, $5 \times 10^5$ amastigotes/well, were added and incubated for 2 h at 37 °C. Pre-diluted plant extracts were subsequently added and the plates were further incubated for 5 days at 37 °C and 5% CO₂. Parasite burdens (mean number of amastigotes/macrophage) were microscopically assessed on 500 cells after Giemsa staining of the testplates, and expressed as a percentage of the blank controls without plant extract.

3.4.5. Antitrypanosomal Activity

*Trypanosoma brucei* Squib-427 strain (suramin-sensitive) was cultured at 37 °C and 5% CO₂ in Hirumi-9 medium [34], supplemented with 10% fetal calf serum (FCS). About $1.5 \times 10^4$ trypomastigotes/well were added to each well and parasite growth was assessed after 72 h at 37 °C by adding resazurin [35]. For Chagas disease, *T. cruzi* Tulahuen CL2 (benznidazole-sensitive) was maintained on MRC-5 cells in minimal essential medium (MEM) supplemented with 20 mM L-glutamine, 16.5 mM sodium hydrogen carbonate and 5% FCS. In the assay, $4 \times 10^3$ MRC-5 cells and $4 \times 10^4$ parasites were added to each well and after incubation at 37 °C for 7 days, parasite growth was assessed by adding the alpha-galactosidase substrate chlorophenol red alpha-D-galactopyranoside [36]. The color reaction was read at 540 nm after 4 h and absorbance values were expressed as a percentage of the blank controls.

3.4.6. Cytotoxicity against MRC-5 Cells

MRC-5 SV2 cells were cultivated in MEM, supplemented with L-glutamine (20 mM), 16.5 mM sodium hydrogen carbonate and 5% FCS. For the assay, $10^4$ MRC-5 cells/well were seeded onto the test plates containing the pre-diluted sample and incubated at 37 °C and 5% CO₂ for 72 h. Cell viability was assessed fluorimetrically after 4 h of addition of resazurin. Fluorescence was measured (excitation 550 nm, emission 590 nm) and the results were expressed as % reduction in cell viability compared to control.

4. Conclusions

Two lupane and four ursane triterpenes were from isolated aerial parts of *K. odora* and identified. Ursane type triterpenes from the biologically active chloroform extract were investigated for their antiprotozoal potential. All were found to have activity against more than one type of the tested parasites, with exception of compound 6.

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**Conflict of Interest**

The authors declare no conflict of interest.

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**Sample Availability**: Samples of the compounds are available from the authors.

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