Scientific paper

In vitro Assessment of Antiprotozoal and Antimicrobial Activities of Fractions and Isolated Compounds from *Pallenis hierochuntica*

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Abstract

The antiprotozoal and antimicrobial properties of the extract and fractions of the whole plant of *Pallenis hierochuntica* were investigated against a panel of pathogenic organisms. Fractionation of the methanol extract of the whole plant of *P. hierochuntica* using reverse-phase chromatography gave 28 fractions and led to the isolation of 2 new bisabolone hydroperoxides, 6,10β,11-trihydroxybisabol-2-ene-1-one (**1a**), 6,10α,11-trihydroxybisabol-2-ene-1-one (**1b**) and also 6,10β-dihydroxybisabol-2,11-diene-1-one (**2**). They were characterised by extensive spectrometric analysis. Anti-infective investigations of the fractions revealed that fractions 22 to 26 possessed significant antimalarial activity against the D6 and W2 strains of *Plasmodium falciparum* with IC_{50} = 7.62–9.91 μg/mL and 5.49–6.08 μg/mL, respectively, and SI > 6.0 on average. Fractions 7, 16 to 24 exhibited good activity against *Leishmania donovani* promastigotes (IC_{50} = 6.71–18.77 μg/mL). Fractions 25 to 28 were active against *Trypanosoma brucei* trypomastigotes, fraction 25 being the most potent (IC_{50} = 4.13 μg/mL). Only fractions 11 to 13 were active against *Aspergillus fumigatus* (IC_{50} = 13.406 μg/mL). Compounds **1a** and **2** were not promising against the organisms tested. **1a** and **1b** were characterised for the first time.

Keywords: *Pallenis hierochuntica*, leishmaniasis, antimalarial, characterization, spectrometry

1. Introduction

Medicinal plants have been a major reservoir of unique and chemically diverse molecules and a large pool of novel drug leads. Plants are known to synthesise potent molecules that exhibit anticancer, anti-infective, anti-inflammatory, antiviral and antiprotozoal activities. Today, many drugs in clinical use are either directly obtained from natural sources or natural products derived. A study conducted by Newman *et al.* revealed that over 35% of drugs approved by the United States in the past four decades are either natural products or their derivatives.[1]

The genus *Pallenis* (synonym: *Asteriscus*) is known to express biologically valuable compounds, especially those with humulene and bisabolone skeletons. Extracts, fractions and isolated compounds from members of this genus have been shown to exhibit different pharmacological activities: antibacterial and antileishmanial,[2,3] anticancer and phytotoxicity,[4] and antioxidant activity.[5] Phytochemical studies of some species of this genus have resulted in the isolation and characterisation of bioactive humulene skeleton sesquiterpene lactones: asteriscunolides A–D[6–9], steriscanolide and aquatolide sesquiterpene lactones,[9] flavonoids, bisabolone hydroperoxides and farsenol derivatives.[10,3,11] Others include sesquiterpene alcohol, germacrane and deoxygenated germacrane,[12,13] and naupliolide, having a novel tetracyclic skeleton.[14]

This study investigated the antiprotozoal and antimicrobial activity of fractions of *Pallenis hierochuntica* (Michon) Greuter, family Asteraceae. Herein we report the isolation, characterisation, and structure elucidation of three compounds (two of these are new) from the methanol extract of the whole plant and their antiprotozoal and antimicrobial activities.

2. Materials and Methods

2. 1. General

Experimental

The acquisition of the 1D and 2D NMR spectra were done on Bruker Avance III 500 and 400 MHz spectrometer. The compounds were dissolved in CD_3OD (^{13}C and ^1H NMR data at 125 and 500 MHz, respectively). Chemical shift values are reported in ppm and referenced to the residual protons of the solvent (CD_3OD). Mass spectra were acquired on an Agilent Technologies 6200 series mass spectrometer. Isolations and purifications of all compounds were performed by column chromatography (CC), over normal silica gel (32–63 μ, Dynamic adsorbents Inc.), and reversed-phase C-18 silica Polar Plus (J. T. Baker®). Analytical TLC was conducted on precoated silica gel F_{254} aluminum sheets (0.25 mm, Sorbtent Tech.) or Silica 60 RP–18 F_{254} aluminum sheets (20 × 20 cm, Merck). Spots were visualized by observing under UV at 254 nm and 365 nm light and by spraying with 1% vanillin (Sigma) in conc. H_2SO_4/EtOH mixture (1:9) followed by heating with a heat gun. All isolation and purification procedures were done by using analytical grade solvents (Fisher chemicals). Pentamidine and amphotericin B (Sigma-Aldrich, St Louis, MO) were used as standard antileishmanial agents. Chloroquine and artemisinin (Sigma-Aldrich, MO) were used as drug controls in the antimalarial assay. Fluconazole, amphotericin B, ciprofloxacin, vancomycin, methicillin, cefotaxime and meropenem were used as positive control antibacterial and antifungi agents.

2. 2. Plant Material

The whole plant of *Pallenis hierochuntica* was collected from the Mediterranean coastal area of Egypt in 2015, and the plant was identified at the Pharmacognosy Department, Mansoura University, Egypt, where a voucher specimen (AH-14-PD) was deposited.

2. 3. Extraction and Isolation

The dried whole plant of *Pallenis hierochuntica* was grounded to powder. The powdered plant material (500 g) was macerated with methanol (98%) by percolation (4 L × 4) for 48 h at room temperature. The solvent was removed with a rotary evaporator at 40 °C to give 35 g of crude extract (7% as yield). The extract (33 g) was mixed with 30 g RP-18 silica gel and applied to a VLC over RP-18 silica (30 cm × 3.5 cm, 500 g) and eluted with gradients of H_2O/MeOH (90:10–0:100) and acetone to give 28 fractions (AH-1 to AH-28). Fraction AH-25 (1.8 g) was subjected to column chromatography (SiO_2, EtOAc:$CHCl_3$:MeOH:H_2O (15:8:4:1; 10:6:4:1; 8:2:0.25; 7:3:0.5; MeOH 100%)) to give 45 sub-fractions AH1A–AH45A. Fractions with similar R_f were pooled to get 7 fractions (H1–H7). Fraction H6 (200 mg) was processed over column chromatography with normal silica gel (3 × 65 cm) eluted with hexane:EtOAc (4:1, 7:3, 3:2) to give 8 fractions (G1–G8). Repeated column chromatographic purification of fraction G8 (40 mg) with hexane:EtOAc (4:1–3:2) yielded AH6 (2.4 mg) as fine needles. Compounds **1a** (0.8 mg) and **1b** (0.7 mg) were purified from AH6, and **2** was purified from PTLC (20 × 20 cm, 500 μm pore size) of AH5 (14.9 mg) with elution system of hexane:chloroform (1:4) (50 mL) to give 0.8 mg of white solid.

2. 4. Antiprotozoal and Antimicrobial Assays

2. 4. 1. Antileishmanial Assay

The fractions and isolated compounds **1a** and **2** were evaluated against *Leishmania donovani* promastigote, *L. donovani* axenic amastigote, and *L. donovani* amastigote in THP1 according to the protocol described by Jain *et al.*[15] which uses the Alamar Blue colourimetric assay method.[16] Pentamidine and amphotericin B standard antileishmanial drugs were used as positive controls. The IC_{50} and IC_{90} values were computed from response curves using XLFit®.

2. 4. 2. Antimalarial Assay

The *in vitro* antiplasmodial activity of the fractions and compounds **1a** and **2** was measured by a colourimetric assay that determines the parasites lactate dehydrogenase (pLDH) activity.[17,18] Included in this assay are two strains of *Plasmodium falciparum* (Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine resistant) obtained from the Walter Reed Army Institute of Research, Silver Spring, MD. The effects of the fractions and test compounds on plasmodial LDH activity were determined using Malstat reagent (Flow Inc, Portland, OR). DMSO (0.25%) and chloroquine/artemisinin were included in each assay which serves as vehicle and positive control drugs, respectively.

2. 4. 3. Cytotoxicity Assay

The cytotoxicity of the test samples was determined against transformed human monocytic (THP1) cells. The assay method previously described by Jain *et al*. was adopted. This experiment used a 4 days old culture of THP1 cells in the experimental phase diluted with RPMI medium to $2.5 \cdot 10^5$ cells/mL. To achieve the parasite cells transformation to the adherent macrophages, Phorbol 12-myristate 13-acetate (PMA) was added to the culture at a concentration of 25 ng/mL. The THP1 cell culture treated with PMA was seeded into 96 well plates with 200 μL culture ($2.5 \cdot 10^5$ cells/mL) in each well and incubated overnight at 37 °C in a 5% CO_2 incubator. The medium in plates with THP1 cells was replaced with a fresh medium. The test samples (fractions and compounds) and standards diluted with RPMI medium in separate plates were added to these plates and then incubated in a 5% CO_2 incubator at 37 °C

for 48 h. After the incubation period, each well received 10 μL of Alamar Blue solution (AbDSerotec, catalogue number BUF012B), and the plates were incubated further overnight. Again, standard fluorescence was measured on a Fluostar Galaxy fluorometer (BMG LabTechnologies) at 544 nm excitation, 590 nm emission wavelengths. The half-maximal concentration IC_{50} and IC_{90} values were computed from the dose-response growth inhibition curve by XLfit version 5.2.2.[15] The selectivity indices (SI) were computed by measuring the cytotoxicity of the test compounds against Vero cell lines (monkey fibroblast).[15]

2. 4. 4. *In vitro* Antimicrobial Activity

Extracts, fractions, and isolated compounds of *P. hierochuntica* were subjected to *in vitro* susceptibility testing against a panel of pathogenic organisms: the fungi include *Candida albicans* (ATCC 90028), *Candida krusei* (ATCC 6258), *Candida glabrata* (ATCC 90030), *Cryptococcus neoformans* (ATCC 90113), *Aspergillus fumigatus* (ATCC 204305); while the bacteria include methicillin-resistant bacterium *Staphylococcus aureus* (MRSA; ATCC 33591), *Escherichia coli* (ATCC 35218), *Klebsiella pneumonia* (ATCC 43816), vancomycin-resistance *Enterococcus faecium* (49532) and *Mycobacterium intracellulare* (ATCC 23068) using a modified version of the NCCLS methods.[19] On the other hand, that against *M. intracellulare* was done using the modified Alamar Blue procedure previously described.[20] The fungi and bacteria used in this experiment were obtained from the American Type Culture Collection (ATCC), Manassas, VA. All the test samples were dissolved in DMSO (0.25%), which also acted as a negative control agent. They were all diluted with 0.9% saline serially and transferred in duplicate to the 96-well microtitre plates. The final microbial inoculums were prepared after comparison of the absorbance at 630 nm of cell suspensions to the 0.5 McFarland standard and diluting the suspensions in broth (Sabouraud dextrose and cation-adjusted Müller–Hinton (Difco) for the fungi and bacteria, respectively, and 5% Alamar Blue (BioSource International) in Middlebrook 7H9 broth to afford recommended inocula. Microbial inocula were added to the diluted samples to realize a final volume of 200 μL. The microtitre plates were read at either 630 nm or 544ex/590em before and after incubation. IC_{50} values relative to controls were obtained using XL fit 4.2 software (IDBS, Alameda, CA).

3. Results and Discussion

3. 1. Characterisation of Compounds 1a and 1b

Compounds **1a** and **1b** were purified from compound **1** (2.4 mg) which showed a clear molecular ion peak $[M+Na]^+$ at *m/z* 293.1696, corresponding to the molecular formula $C_{15}H_{26}O_4Na$ (calcd 293.1729) from its HRESIMS spectrum. However, careful examination of the ^1H and ^{13}C NMR spectra of **1** (Figure S18), revealed the presence of double peaks, suggesting a mixture of two bisabolone-type sesquiterpenoids[3], a feature usually associated with closely related compounds (mixtures).

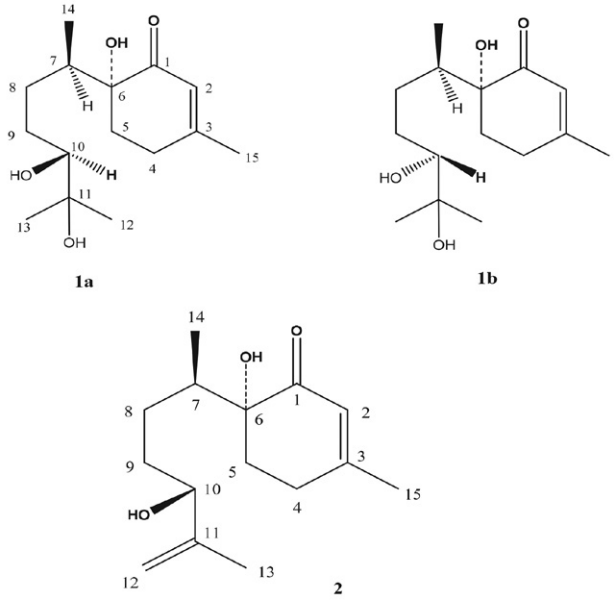

Figure 1. Molecular structures of compounds **1a**, **1b** and **2**

As such, compound **1** was subjected to further purification to give two compounds of the same molecular weight, **1a** (0.8 mg) and **1b** (0.7 mg). Compound **1a** (6,10β,11-trihydroxybisabol-2-ene-1-one), white needles from sub-fraction AH6 (0.8 mg), HRESIMS $[M+Na]^+$ at *m/z* 293.1694, corresponding to the molecular formula $C_{15}H_{26}O_4Na$ (calcd 293.1729). The ^1H NMR spectrum of **1a** in CD_3OD (Table 1) displayed an olefinic proton signal at δ 5.84 (1H, *dq*, *J* = 2.4, 1.1 Hz), a doublet at δ 1.01 (3H, *d, J* = 6.7 Hz), a singlet at δ (6H, *s*, 1.12), a singlet at δ 1.99 (3H, *s*) and a methine proton at δ 3.15 (*dd*, *J* = 9.3, 2.4 Hz). The ^{13}C NMR spectrum (Figure S2) revealed the presence of characteristic signals: a carbonyl carbon at δ 204.1, olefinic methine at δ 124.5 and quaternary olefinic carbon at δ 165.5, together with the olefinic proton signal at δ 5.84 (1H, *dq*, *J* = 2.4, 1.1 Hz), indicated the presence of a monosubstituted α,β-unsaturated carbonyl groups. The DEPT experiment (Supplementary material, Figure S4) showed the presence of four methylene carbons at δ 31.6, 29.1, 28.9 and 29.3. The HMQC (Supplementary material, Figure S6) revealed their connectivities with the proton signals at δ (2.24, 2.28), (1.29, 1.40), (1.06, 1.27), and (2.42, 2.44), respectively. The correlations observed in HMBC spectrum (Supplementary material, Figure S7) between the proton at δ 2.36 with carbon signals at δ 125.0, 165.5, 32.9, and 78.1 (quaternary carbon), together with the correlations be-

tween the protons at δ 2.24 with 78.1 (quaternary carbon), 202.6 and 29.1, and between the proton at δ 5.84 with 78.1 and 29.1, are consistent with the cyclic (six-carbon ring) unsaturated ketone with branching at the oxygenated quaternary carbon (δ 78.1). The side chain consists of eight carbon atoms discriminated by DEPT experiment into three methyls (δ 24.3, 23.5 and 12.6), two methylene (δ 28.7.0 and 27.4), one quaternary (δ 72.4), and one methine (δ 78.8). Careful examination of all correlations in $^{1}H–^{1}H$ COSY, HMQC and HMBC confirmed the proposed identity of the structure, which is a bisabolone-skeleton with an OH at C-10 (δ 78.8) and C-11 (δ 72.4) (Figure 1). The relative configuration of **1a** was established through the analysis of cross-peaks observed in the NOESY spectrum (Supplementary material, Figure S8), which displayed a correlation of H-15 (*s*, 1.99, 3H) with both H-14 (*d*, 1.01, 3H) and H-4 (2.44), correlation of H-10 (*dd*, 3.16) with H-14 (*d*, 1.01, 3H), indicated the β-oriented H-14 and H-10. Therefore, the structure of compound **1a** was determined to be 6,10β,11-trihydroxybisabol-2-ene-1-one. The other compound **1b** (6,10α,11-trihydroxybisabol-2-ene-1-one), white needles from sub-fraction AH6 (0.7 mg), HRESIMS $[M+Na]^+$ at *m/z* 293.1694, corresponding to the molecular formula $C_{15}H_{26}O_4Na$ (calcd 293.1729), was of the same structure as **1a** with a slight deviation in the NMR signals (Table 1, supplemental material, Figures S11–S17). The differences resulted from the hydroxy group orientation at C-10. Upon careful analysis of the NOESY (Supplementary material, Figure S16) spectrum of **1b**, the correlation of H-15 (*s*, 1.99, 3H) with both H-14 (*d*, 1.02) and H-4 (2.44), and the correlation of H-10 (*dd*, 3.15) with H-7 (*m*, 1.91), indicated that the 10-OH is α-oriented. The structure of **1b** was concluded to be 6,10α,11-trihydroxybisabol-2-ene-1-one.

3. 2. Characterisation of Compound 2

Compound **2** (Figure 1) was obtained as fine white needles. HRESIMS showed a clear molecular ion peak $[M+Na]^+$ at *m/z* 275.1594 (calcd $C_{15}H_{24}O_3Na$, 275.1623). The ^{1}H and ^{13}C NMR spectra of **2** revealed the same skeleton as compound **1a**, a bisabolone-type sesquiterpene, with the disappearance of the one methyl and oxygenated quaternary carbon at δ 72.5 which was assigned for C-11 of **1a** and **1b**, and the appearance of one olefinic methylene group and quaternary olefinic carbon. The ^{1}H NMR spectrum of **2** showed two olefinic protons at δ 4.86 (*p*, *J* = 1.6 Hz) and 4.87 (*dt*, *J* = 1.9, 0.9 Hz) (Table S1) assigned to C-12 explaining the absence of a methyl group. The ^{13}C NMR displayed a hydroxylation at C-10, the same as compound **1**a, and the H-10 chemical shift at δ 3.94 together with the coupling value (*t*, *J* = 6.7 Hz) is in full agreement with the β-oriented C-10 hydroxyl group. The chemical structure of **2** was deduced as 6,10β-dihydroxybisabol-2,11-diene-1-one (Figure 1). Compound **2** was previously reported as a reduction product of 10-peroxy derivatives,[3] but this is the first time it was isolated directly from a natural source.

3. 3. *In vitro* Antiparasitic Screening of Fractions and Isolated Compounds

The results of the antimalarial screening of the fractions against the two strains of *Plasmodium falciparum* (D6 and W2) are shown in Table S2 (Supplementary material). Similarly, the antileishmanial and antitrypanosomal results of the fractions are shown in Table S3 (Supplementary material), and the antimicrobial activity of the fractions is reported in Table S4. Results of the *in vitro* antimalarial, antileishmanial, antitrypanosomal and antimicrobial activities of compounds **1a** and **2** are presented in Tables S5 to S7 (Supplementary material).

According to the WHO report of 2019, it was estimated that there were 229 million cases of malaria worldwide, resulting in 409,000 deaths, most of whom are children under five, especially in Sub-Saharan Africa, making malaria a major global health problem.[21] Several researchers have reported the antimalarial effects of plant extracts and fractions.[22–24] According to the WHO, about 80% of people depend on herbal products as their primary healthcare source(s).[21]

The result of the antimalarial screening of the fractions of *Pallenis hierochuntica* is shown in Table S2 (Supplementary material). In the primary screening experiment, the two strains (D6 and W2) of *P. falciparum* were tested against the fractions at concentrations range of 47.6–5.28 μg/mL. Only fractions that showed antimalarial activity (≥ 50%) in this screening were investigated in the secondary antimalarial screening to determine their IC_{50} values. From the table, fractions AH11–AH12 and AH14–AH28 exhibited significant antimalarial activity against the chloroquine-sensitive (D6) and resistant (W2) strains of *P. falciparum* with IC_{50} values ranging from 7.62–30.33 μg/mL and 5.49–25.49 μg/mL, respectively. Fractions AH23–AH27 were particularly effective against the two strains of PF with IC_{50} = 5.49–9.19 μg/mL. As such, these fractions are classified as having promising antimalarial activity. Their IC_{50} values were, however, higher than those obtained for artemisinin and chloroquine (IC_{50} <0.026–0.202 μg/mL), standard antimalarial drugs used as positive control drugs. They also showed better selectivity indices (SI = > 5.2 – > 8.7). Our report is the first on the *in vitro* antimalarial activity of this plant. There is a lack of information on the antimalarial activity of the *Pallenis* genus representatives in general.

Similarly, leishmaniasis and trypanosomiasis (sleeping sickness) affect humans and livestock in the tropical and subtropical countries of Africa, Asia and South America. It has been estimated that over 70 million and 350 million people worldwide are at risk of trypanosomiasis and leishmaniasis, respectively, with attendant annual deaths of 14,000 to 70,000.[25] In our continued investigation of

Table 1. ^{13}C and ^{1}H NMR data for compound **1a** and 1**b** (CD_3OD at 125 and 400 MHz, respectively).

Carbon No.	1a			1b		
	δ_H (ppm), Multiplicity, *J* (Hz)	^{13}C	DEPT	δ_H (ppm), Multiplicity, *J* (Hz)	^{13}C	DEPT
1	--	204.1	C	--	204.0	C
2	5.84 (*dq*, *J* = 2.4, 1.1 Hz)	123.2	CH	5.83 (*dq*, *J* = 2.4, 1.1 Hz)	124.6	CH
3	--	163.9	C	--	165.2	C
4	2.42 2.44	29.3	CH_2	2.36 2.44	30.5	CH_2
5	2.24(*ddd*, J = 5.0, 3.3, 1.7Hz) 2.28	31.6	CH_2	2.23(*ddd*, J = 5.0, 3.3, 1.7 Hz) 2.28	33.1	CH_2
6	--	78.1	C	--	78.5	C
7	1.91, *m*	35.4	CH	1.91, *m*	37.3	CH
8	1.06 1.27	28.9	CH_2	1.07 1.26	29.0	CH_2
9	1.29 1.40	29.1	CH_2	1.29 1.40	30.8	CH_2
10	3.15 (*dd*, *J* = 9.3, 2.4 Hz)	78.8	CH	3.15 (*dd*, *J* = 9.3, 2.4 Hz)	80.3	CH
11	--	72.5	C	--	74.0	C
12 13	1.12, *s*	24.3	CH_3 CH_3	1.14, *s*	25.9	CH_3 CH_3
14	1.01(*d*, *J* = 6.7 Hz)	12.5	CH_3	1.02 (*d*, *J* = 6.7 Hz)	14.1	CH_3
15	1.99, *s*	23.5	CH_3	1.97, *s*	23.9	CH_3

medicinal plants for antiprotozoal metabolites, fractions of *Pallenis hierochuntica* were subjected to *in vitro* screening against *Leishmania donovani* (promastigotes, axenic amastigotes, and intracellular amastigotes in THP1 cells) and blood-stage promastigotes of *Trypanosoma brucei*. The result of this screening is shown in Table S3 (Supplementary material). From the result, fractions AH-1 and AH-3 showed activity against *T. brucei,* IC_{50} = 11.33 and 12.50 μg/mL, respectively. Fraction AH-7 was active against *L. donovani* promastigotes and axenic amastigotes with IC_{50} and IC_{90} values of 12.03–19.47 μg/mL and also against *T. brucei* (IC_{50} = 13.6 μg/mL). Fractions AH-12, AH-16 to AH-18, and AH-24 were also effective against the promastigotes of *L. donovani*. While fractions AH-25 to AH-28 were active against *T. brucei* blood-stage trypomastigotes with IC_{50} values of 4.13–13.48 μg/mL and IC_{90} values of 11.46–19.28 μg/mL. All the fractions at 20–8 μg/mL test concentrations did not show activity against intracellular amastigotes in THP1 cells. The positive control drugs, pentamidine and DMFO, possess better activity against these protozoa except fraction AH-25, which showed better activity (IC_{50} = 4.13 μg/mL) than DMFO (IC_{50} = 6.25 μg/mL) against *T. brucei*. Several studies have highlighted the activity of plant extracts and fractions against leishmaniasis and trypanosomiasis.[26,25,27] Further purification of these extracts and fractions has led to the isolation of potent compounds exhibiting significant leishmanicidal and trypanocidal effects against these pathogenic protozoal.[28–30] In a related study, the ethyl acetate extract of *Asteriscus graveolens* exhibited potent activity against both promastigote and amastigote forms of *L. infantum* and *L. major* with IC_{50} value of 22.93 ± 0.39 μg/mL and 131.6 ± 0.21 μg/mL against *L. infantum.* Also, the hydroethanolic extract of the plant inhibited *L. major* and *L. infantum* parasites with IC_{50} = 33.64 ± 0.46 μg/mL and 143.4 ± 0.28 μg/mL, respectively.[2] An *in vitro* antiprotozoal activity of crude methanol extract of *Pallenis hierochuntica* (*Asteriscus hierochuntica*) was reported by Zaki *et al*.,[31] in which the extract showed promising and good antitrypanosomal activity against the promastigotes of *T. brucei* with IC_{50} and IC_{90} values being 1.18 and 1.89 μg/mL, respectively. Our study is the first report of the antileishmanial and antitrypanosomal activity of the fractions of *Pallenis hierochuntica.*

In the antimicrobial screening experiment, 28 fractions (AH-1 to AH-28) were subjected to *in vitro* antimicrobial evaluation against a panel of pathogenic microorganisms (fungi and bacteria) (Table S4). The fractions were tested at a 200–8 μg/mL concentration range. From the results of our study, only fractions AH-11 to AH-13 exhibited significant activity against *Aspergillus fumigatus* with IC_{50} values of 13.406 (AH-11), 88.607 (AH-12)

and 130.228 μg/mL (AH-13), respectively. Other studies reported the antimicrobial activities of members of the genus Asteriscus. Ramdane *et al.* reported the antimicrobial activity of the ethyl acetate fractions of *A. graveolens* against *L. monocytogenes* (MIC = 0.312 mg/mL), *S. aureus* and *B. cereus* (MIC = 0.625 mg/mL), but the fractions show no activity against *E. coli* (ATCC 35214) and *P. aeruginosa* (ATCC 27853).[2] Also, Medimagh *et al.* evaluated the root oil of *Asteriscus maritimus* (L.) for antimicrobial activity against some pathogenic fungi, including *Aspergillus flavus*, *A. niger*, *Botrytis cinerea* and *Penicillium* sp. The zones of inhibition range from 8.3 mm to 10.3 mm. However, the oil was not active against the bacteria isolates tested.[32] The oil of *A. graveolens* was also reported to significantly (p <0.05) inhibit the mycelial growth of some pathogenic fungi (*Alternaria sp.*, *P. expansum,* and *R. stolonifer*) at different concentrations.[33]

3. 4. *In vitro* Antimalarial and Antimicrobial Activities of Compounds 1a and 2

The *in vitro* antimalarial, antileishmanial, antitrypanosomal and antimicrobial screening of compounds **1a** and **2** are reported in Tables S5–S7 (Supplementary material). They were screened against the different pathogenic organisms mentioned in section 2.4 above. The study results showed that the compounds exhibited no significant activity against tested organisms at the concentrations tested.

4. Conclusion

In the present study, two new compounds with bisabolone skeleton and a known compound were isolated and identified as 6,10β,11-trihydroxybisabol-2-ene-1-one, 6,10α,11-trihydroxybisabol-2-ene-1-one, and 6,10β-dihydroxybisabol-2,11-diene-1-one, respectively. These compounds hold no promising antiprotozoal and antimicrobial activities. The fractions of *Pallenis hierochuntica* show significant activity against *Plasmodium falciparum, Leishmania donovani* promastigotes, axenic amastigotes and trypomastigotes of *Trypanosoma brucei.* This plant holds potential for further investigation for lead compounds as antiprotozoal agents.

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Povzetek

Za ekstrakte iz celotne rastline *Pallenis hierochuntica* in njegove frakcije smo s serijo testov preverili morebitno aktivnosti proti patogenim organizmom (delovanje proti protozoam in proti mikrobom). Frakcioniranje metanolnega ekstrakta iz rastline *P. hierochuntica* s pomočjo reverznofazne kromatografije je dalo 28 frakcij in omogočilo izolacijo dveh novih bisabolonskih hidroperoksidov: 6,10β,11-trihidroksibisabol-2-en-1-ona (**1a**) ter 6,10α,11-trihidroksibisabol-2-en-1-ona (**1b**) in tudi 6,10β-dihidroksibisabol-2,11-dien-1-ona (**2**). Vse tri spojine smo karakterizirali z obširno spektroskopsko analizo. Izkazalo se je, da imajo frakcije 22 do 26 občutno antimalarijsko delovanje proti sevoma *Plasmodium falciparum* D6 (z IC_{50} vrednostmi 7.62–9.91 μg/mL) in W2 (z IC_{50} vrednostmi 5.49–6.08 μg/mL); indeks selektivnosti je bil v povprečju večji od 6.0. Frakcije 7 in 16 do 24 so izkazale dobro aktivnost proti *Leishmania donovani* promastigotom (IC_{50} = 6.71–18.77 μg/mL). Frakcije 25 do 28 so bile aktivne proti *Trypanosoma brucei* tripomastigotom, od katerih se je frakcija 25 izkazala kot najbolj učinkovita (IC_{50} = 4.13 μg/mL). Proti *Aspergillus fumigatus* so bile učinkovite zgolj frakcije 11 do 13 (IC_{50} = 13.406 μg/mL). Izkazalo se je, da spojini **1a** in **2** nista obetavni učinkovini proti testiranim organizmom. Karakterizaciji spojin **1a** in **1b** v literaturi še nista bili opisani.