Published in final edited form as:
Planta Med. 2011 September ; 77(14): 1639–1643. doi:10.1055/s-0030-1270906.

Antiparasitic and Antimicrobial Indolizidines from the Leaves of *Prosopis glandulosa* var. *glandulosa*†

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Abstract

A new indolizidine alkaloid, named $\Delta^{1,6}$-juliprosopine (**1**), together with previously known indolizidine analogs (**2**–**6**), was isolated from the leaves of *Prosopis glandulosa* var. *glandulosa*, collected from Nevada, USA; while two other known indolizidines juliprosopine (**6**) and juliprosine (**7**) were isolated from *P. glandulosa* leaves collected in Texas, USA. The structures of compound **1** and **7** were determined using a combination of NMR and MS techniques. Compound **7** exhibited potent antiplasmodial activity against *Plasmodium falciparum* D6 and W2 strains with IC_{50} values of 170 and 150 ng/mL, respectively, while **1** was found to be less active (IC_{50} values 560 and 600 ng/mL). Both the compounds were devoid of VERO cells toxicity up to a concentration of 23800 ng/mL. The antileishmanial activity of indolizidines was evaluated against *Leishmania donovani* promastigotes, axenic amastigotes and amastigotes in THP1 macrophage cultures. When tested against macrophage cultures, the tertiary bases (**1**, **3**, **6**) were found to be more potent than quaternary salts (**2**, **5**, **7**), displayed IC_{50} values between 0.8–1.7 μg/mL and 3.1–6.0 μg/mL, respectively. In addition, compound **7** showed potent antifungal activity against *Cryptococcus neoformans* and antibacterial activity against *Mycobacterium intracellulare*, while **1** was potent only against *C. neoformans* and weakly active against other organisms.

Keywords

Prosopis glandulosa; Fabaceae; indolizidine alkaloid; $\Delta^{1,6}$-juliprosopine; antimicrobial; antiparasitic

Introduction

Prosopis glandulosa Torrey var. *glandulosa* (Fabaceae), commonly known as Honey Mesquite, is a medium-sized flowering tree native to the southwestern United States and Mexico [1, 2]. The plant is a folk remedy for dyspepsia, eruptions, eyes, hernias, skin ailments, sore throat and umbilical ailments [3, 4]. Earlier investigations on this plant reported various types of biological activities such as antibacterial [5, 6], antifungal [7], anti-

†Part III. For part II, see Samoylenko et al., (2009).

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Supporting information
The isolation and purification of compounds, ^{1}H, ^{13}C NMR and HMBC spectra for compound **1**, and antileishmanial macrophage amastigote assay are available in Supporting Information.

infective and antiparasitic activities [8, 9], which are attributed to piperidinyl indolizidine alkaloids. We have previously reported [8] several potent antiparasitic and antimicrobial diastereoisomeric tertiary and quaternary indolizidine alkaloids from *P. glandulosa* collected in Nevada. In continuation of our previous investigation, we now have isolated a new tertiary indolizidine alkaloid, named $\Delta^{1,6}$- juliprosopine (**1**), and large amounts of five known indolizidines (**2**–**6**) [8]. In addition, we also have examined a sample of *P. glandulosa* var. *glandulosa*, obtained from Texas, which yielded two known indolizidines, namely the tertiary juliprosopine (**6**) and the quaternary juliprosine (**7**), and interestingly devoid of compounds **1**–**5**. We herein report the isolation and structure elucidation of **1**, as well as the antiplasmodial and antimicrobial activities of **1** and **7**, and a detailed antileishmanial activity of isolated compounds (**1**–**3**, **5**–**7**) against *Leishmania donovani* promastigotes, axenic amastigotes and amastigotes in THP1 macrophage cultures.

Materials and Methods

General

Optical rotations were measured in $CHCl_3$ or MeOH using an AUTOPOL IV instrument at ambient temperature. IR spectra were obtained using a Bruker Tensor 27 instrument. NMR spectra were acquired on a Varian Mercury 400 spectrometer (Varian Inc.; Palo Alto, CA) with standard pulse sequences operating at 400 MHz in ^{1}H NMR and 100 MHz in ^{13}C NMR in $CDCl_3$ or CD_3OD (Cambridge Isotope Laboratories, Inc.) using the residual solvent as internal standard. TLC was carried out on aluminum oxide IB-F plates (Baker-flex) using CH_2Cl_2-MeOH-NH_3·H_2O (8:2:0.1) as solvent. Centrifugal preparative TLC (CPTLC, Chromatotron®, Harrison Research Inc., model 8924) was carried out on 1, 2 and 4 mm alumina (Analtech, Inc.) rotors, using CH_2Cl_2-MeOH-NH_3·H_2O as solvent. The compounds were visualized by observing under UV light at 254 or 365 nm, followed by spraying with Dragendorff's or 1% vanillin-H_2SO_4 spray reagents. All positive controls for biological experiments are commercially available with declared purity from known vendors.

Plant Material

The leaves of *P. glandulosa* var. *glandulosa* were collected from Nevada, USA (voucher # NIX 230506/1/A) in May 2006 and Texas (voucher # 3794/11062008/PRGLM) in June 2008, and were identified by Dr. Vaishali C. Joshi, NCNPR, School of Pharmacy, University of Mississippi. Voucher specimens are deposited at the Herbarium of NCNPR, University of Mississippi.

Extraction and isolation

The air dried powdered leaves of *P. glandulosa* (Nevada, 1.8 kg) were extracted by percolation (95% EtOH, 2 L × 3, 48 h) and resulted EtOH extracts was evaporated to dryness (273 g). The dried extracts (130 g) was dissolved in aqueous 0.1 N HCl and partitioned successively with *n*-hexane (1L × 3) followed by CH_2Cl_2 (600 mL × 3). The aqueous acidic layer was then basified with 0.1 N NH_4OH to pH 11 (based on pKa 10.2 of tryptamine), followed by partitioning with CH_2Cl_2 (500 mL × 4). The pH of the remaining basic layer was then adjusted to 12 by NH_4OH, followed by partitioning successively with CH_2Cl_2 (500 mL × 3) and EtOAc (300 mL × 3). The CH_2Cl_2 fraction (2.8 g) was subjected to CPTLC, using 4 mm Al_2O_3 rotor with CH_2Cl_2-MeOH-NH_3 ·H_2O as solvent to yield 13 fractions. Further purification by repeated CPTLC afforded compound **1** (37 mg), **2** (60 mg), **3** (53 mg), **4** (35 mg) **5** (94 mg) and **6** (84 mg) (Fig. 4S in Supporting Information). Compounds **2**–**6** were identified by ^{1}H and ^{13}C NMR spectral data, as well as by direct comparison with authentic samples of prosopilosidine, prosopilosine, isoprosopilosine, isoprosopilosidine and juliprosopine, respectively, previously isolated from the same plant [8].

A sample of *P. glandulosa* (1 Kg, air dried) leaves collected in Texas was extracted (95% EtOH, 1.5 L × 3, 48 h) and evaporated to dryness (yield 149 g). The EtOH extract was fractionated using acid/base treatment to give alkaloidal enriched fraction (67 g), and then processed according to procedure as described previously [8]. The TLC characteristic of alkaloidal fraction was found to be significantly different to those of the samples from Nevada. CPTLC separation of alkaloidal fraction, using above method, yielded compound **6** (200 mg) and juliprosine (**7**; 225 mg). The identity of compound **6** was established by ^{1}H and ^{13} NMR spectral data and direct comparison with an authentic sample of juliprosopine.

$\Delta^{1,6}$-juliprosopine (1)—Colorless gum; $[\alpha]^{20}_{D}$ +6.9 (MeOH, c 0.5), R_f 0.63 [Al_2O_3/CH_2Cl_2-MeOH-NH_3·H_2O (96:4:0.1)]; UV (CH_3OH) λ_{max} (log ε) 287.0 (2.81), 204.5 (3.48); IR (film) ν_{max}, cm^{-1} 3270 (OH, NH), 2924, 2852, 1462 (C-H) and 1657 (C=N); ^{1}H and ^{13}C NMR, see table 1; HRESIMS *m*/*z* 626.5628 $[M + H]^+$ (calcd for $C_{40}H_{71}N_3O_2$, 626.5624).

Juliprosine (7)—Colorless gum; $[\alpha]^{20}_{D}$ +5 ($CHCl_3$, c 0.5) [Lit. 21: $[\alpha]_D$ 11, $CHCl_3$], R_f 0.22 [Al_2O_3/CH_2Cl_2-MeOH-NH_3·H_2O (96:4:0.1)]; UV (CH_3OH) λ_{max} (log ε) 277 (3.81), 226 (3.79), 222 (3.85); IR (film) ν_{max}, cm^{-1} 3291 (OH, NH), 2925, 2853, 1458 (C-H), and 1506 (C-C); The ^{1}H and ^{13}C NMR spectral data were in agreement with those reported previously by Daetwyler *et al.* [21], HRESIMS: *m*/*z* 628.5854 $[M]^+$ (calcd. for $C_{20}H_{27}O_4$, *m*/*z* 628.5854).

Biological activity

Antileishmanial assay—The *in vitro* antileismanial assay was done on a culture of *Leishmania donovani* promastigotes and axenic amastigotes by Alamar Blue assay [13]. In a 96 well microplate the samples with appropriate dilution were added to the leishmania promastigotes/axenic amastigote culture (2×10^6 cell/mL). The compounds were tested in duplicates at six concentrations ranging from 40 to 0.0128 μg/mL. The plates were incubated at 26 °C for 72 hours (37 °C for amastigote) and growth of leishmania promastigotes/amastigote was determined. IC_{50} and IC_{90} values were computed from the dose response curves. The *in vitro* amastigote assay in macrophages is described in Fig. 5S, Supporting Information Section. The compounds were also tested against VERO cells and PMA transformed THP1 cells by Neutral Red assay [22].

Antiplasmodial assay—The *in vitro* antiplasmodial activity, as described previously [8], was measured by a colorimetric assay that determines the parasitic lactate dehydrogenase (pLDH) activity [14]. All experiments were carried out in duplicates.

Antimicrobial assay—All microorganisms were obtained from the ATCC (Manassas, VA) and include the fungi *Candida albicans* (ATCC 90028), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC 90030), *Cryptococcus neoformans* (ATCC 90113), and *Aspergillus fumigatus* (ATCC 204305) and the bacteria *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *S aureus* (MRSA, ATCC 33591), and *Mycobacterium intracellulare* (ATCC 23068). For all organisms, excluding *M. intracellulare* and *A. fumigatus*, susceptibility testing was performed using a modified version of the CLSI (formerly NCCLS) methods [15, 16] and optical density was used to monitor growth. Media supplemented with 5% Alamar Blue (BioSource International, Camarillo, CA) was utilized for growth detection of *M. intracellulare* [17] and *A. fumigatus* [18]. Concentrations that afford 50% inhibition (IC_{50}s) relative to controls were calculated using XLfit 4.2 software (IDBS, Alameda, CA) using fit model 201 based on duplicate readings. Minimum fungicidal or bactericidal concentrations were determined by removing 5 μL from each clear well, transferring to agar, and incubating until growth was seen. Drug controls [Ciprofloxacin

(ICN Biomedicals, Ohio, 99.3% purity) for bacteria and Amphotericin B (ICN Biomedicals, Ohio, 94.8% purity) for fungi] were included in each assay.

Results and Discussion

Compound **1** (colorless gum) was analyzed for a molecular ion of *m*/*z* 626.5628 $[M+H]^+$ (calcd. 626.5624) by HRESIMS for $C_{40}H_{71}N_3O_2$. The IR (film) spectrum showed the presence of OH/NH group(s) (ν_{max} 3270 cm^{-1}) and C=N (ν_{max} 1657 cm^{-1}) absorption bands. The NMR spectra of **1** revealed the presence of a 6,7-dehydroindolizidine nucleus [10, 11, 19, 20] consisting of four triplets, three doublets, and a singlet (Table 1, Fig. 2S in supporting information). In addition, NMR spectra displayed oxymethine, methine adjacent to nitrogen, methylene, and methyl signals at δ_C 66.3, 56.1, 25.6, 29.5, and 17.5, respectively, assigned to two piperidinyl rings, as well as 10 methylenes for two decanyl moieties. Comparison of NMR spectra of **1** with spectra of other dehydroindolizidines such as juliprosopine [8, 12] suggested close similarities with that of juliprosopine (**6**). However, there is only one notable difference for the piperidinyl rings of **1** (δ_C 56.1, 66.3, 25.6, 29.5, 171.1, and 17.5; C-2/2′-C-7/7′) with that of juliprosopine (δ_C 57.2, 67.8, 26.1, 25.8, 55.8, and 18.4; C-2/2′-C-7/7′) at C-6/6′. The downfield value observed for C-6/6′ (δ_C 171.1, quaternary carbon) when compared with juliprosopine (δ_C 55.8, tertiary carbon) suggested the presence of a double bond between 1 and 6 position (nitrogen and carbon), which allowed us to conclude that the piperidinyl ring was dehydrogenated at this position. The HMBC experiment of **1** (Table 1) showed correlations between H-7‴′ and C-5‴′, C-8‴′, C-8a‴′, and C-10‴; H-10‴ and C-5‴′, C-6‴′, and C-7‴′; H-8‴ and C-6‴′, C-7‴′, and C-8a‴′ confirming a 6,8-dialkylated 6,7-dehydroindolizidine nucleus of juliprosopine (**6**). The NOESY experiment showed correlation between H-2/2′ and H-3/3′, suggesting that these protons were *cis* and on the α-face of the molecule, thereby the C-2/2′ methyl group and C-3/3′ OH group were on the β face (3*R*) of the molecule, hence two 2β-methyl-3β-hydroxy-6-decanylpiperidine moiety with the same configuration for both ring was confirmed. On the basis of above discussion, the structure of **1** was deduced as shown (Figure 1), and it was named as $\Delta^{1,6}$-juliprosopine.

During the course of isolation of **1**, five other indolizidines **2**-**6** (Fig. 1) were isolated from *P. glandulosa* from Nevada. Their structures were confirmed by NMR [8] and direct comparison with authentic samples (available in our laboratory) as prosopilosidine (**2**), prosopilosine (**3**), isoprosopilosine (**4**), isoprosopilosidine (**5**) and juliprosopine (**6**). Examination of EtOH extracts of *P. glandulosa* leaves, collected from Texas, showed weak *in vitro* antiinfective and antiparasitic activities. Its alkaloidal profile, by TLC, was found to be substantially different with those observed for Nevada sample. This prompted us to carry out an acid/base fractionation. The bioactivity of the EtOH extract was significantly increased in the alkaloid enriched CH_2Cl_2 fraction. A CPTLC of the CH_2Cl_2 fraction resulted in the isolation of compounds **6** [8] and **7** [21], in the yields of 0.044% and 0.050%, respectively. The structure of compound **7** was confirmed by comparing its physical and spectral data (^1H and ^{13}C NMR and MS) with those reported previously for juliprosine [21].

$\Delta^{1,6}$-juliprosopine (**1**) and juliprosine (**7**) were tested for *in vitro* antiplasmodial, antileishmanial, antibacterial and antifungal activities. The dihydroindolizinium salt (**7**) exhibited potent antiplasmodial activity and high SI (Selectivity Index) (Table 2) against *P. falciparum* (IC_{50} values of 170 and 150 ng/mL, against chloroquine sensitive (D6) and chloroquine resistant (W2) strains, respectively). The activity of **7** was found to be similar to those of chloroquine (IC_{50}= 150 and 140 ng/mL) against *P. falciparum* W2 strain. However, the antiplasmodial activity of **7** was found to be less potent compared to its diastereoisomer prosopilosidine (**2**), previously isolated from same plant [8]. On the other hand, the dehydroindolizidine base $\Delta^{1,6}$-juliprosopine (**1**) was less potent (IC_{50} = 560 and 600 ng/mL

against D6 and W2 strains) than **7** and juliprosopine (**6**) [8], with no toxicity against mammalian VERO cells like **7**, and less toxic than **6** (IC_{50} = >23800 ng/mL for both **1** and **7** *vs*. 5000 ng/mL for **6**). Therefore, **2** and **7** exhibited higher SI's against *P. falciparum* D6 and W2 strains (SI >610, >250 and SI >140, >159, respectively) than **1** and **6** (SI >43, 23 and SI 40, 13 respectively), confirming our previous observation that the quaternary alkaloids (i.e., **2**, **7**) are better candidates than the tertiary bases (i.e., **1**, **6**) for further antiplasmodial studies.

The antileishmanial activities of tertiary (**1**, **3**, **6**) and quaternary (**2, 5, 7**) indolizidines were evaluated against promastigotes and axenic amastigotes [13], and amastigotes in macrophage cultures of *L. donovani* (Table 3). The promastigotes/amastigotes activity of **3** and **6** were found to be equally potent to standard drug pentamidine (IC_{50}= 0.18/1 and 0.3/2 μg/mL for **3** and **6** *vs.* 0.8/2.1 μg/mL for pentamidine). However, the quaternary salts (**2**, **5** and **7**) were found to be less potent, but also less toxic towards VERO cells than tertiary bases (**3** and **6**). Interestingly, the presence of a C-1,2 double bond in both piperidine rings, as observed in **1**, retained potent antileishmanial activity (IC_{50}= 0.8 and 1.8 μg/mL) against both *L. donovanii* promastigotes and axenic amastigotes, respectively, while decreased its VERO toxicity, compared to **3** and **6**. When tested against *L. donovanii* in THP1 macrophage cultures, the tertiary bases (**1**, **3**, **6**) were found to be more potent than quaternary salts (**2**, **5**, **7**), displayed IC_{50} values between 0.8-1.7 μg/mL *vs*. 3.1–6.0 μg/mL. The activities of tertiary bases in THP1 were also superior to those of pentamidine (IC_{50} = 3.0 μg/mL).

Finally, compound **7** showed potent *in vitro* antifungal activity against *C. neoformans*, and antibacterial activity against *M. intracellulare* with IC_{50}/MIC values of 0.17/0.63, and 0.80/1.25 μg/mL, and **1** against *C. neoformans* (IC_{50}/MIC = 0.66/1.25 μg/mL, respectively) (Table 4). The minimum fungicidal concentration (MFC) of **7** against *C. neoformans* was equipotent to amphotericin B. Both the compounds showed weak activities against *S. aureus* and MRSA, while **1** also displayed weak activity against *C. albicans*, *C. krusei* and *M. intracellulare*. The antifungal activity of a mixture of juliprosine (**7**) and isojuliprosine were reported previously against various pathogenic fungi [7], however juliprosine had not been reported previously for biological activity as a single pure compound.

This appears to be the first report of $\Delta^{1,6}$-juliprosopine (**1**) from either a natural or synthetic sources. Compound **1** is a new addition to our previously reported indolizidines (**2–6**) [8] from *P. glandulosa*, collected in Nevada. During this investigation, we also analyzed *P. glandulosa* sample from Texas, which afforded only **6** and **7** and devoid of indolizidines **1**–**5**. This work suggests that the qualitative and quantitative nature of the bioactive alkaloidal profile in *P. glandulosa* varies significantly due to geographical location.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors sincerely thank Dr. Mark Blumenthal, American Botanical Council, and Mr. Elray Nixon for supplying plant material from Texas and Nevada, respectively, Dr. Bharathi Avula, NCNPR, for recording mass spectra, and Ms. Marsha Wright and Mr. John Trott for assistance in biological work. This work was supported by US Department of Defense CDMRP grant # W81XWH-09, and in part by USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-0009 and NIH, NIAID, Division of AIDS, Grant No. AI 27094.

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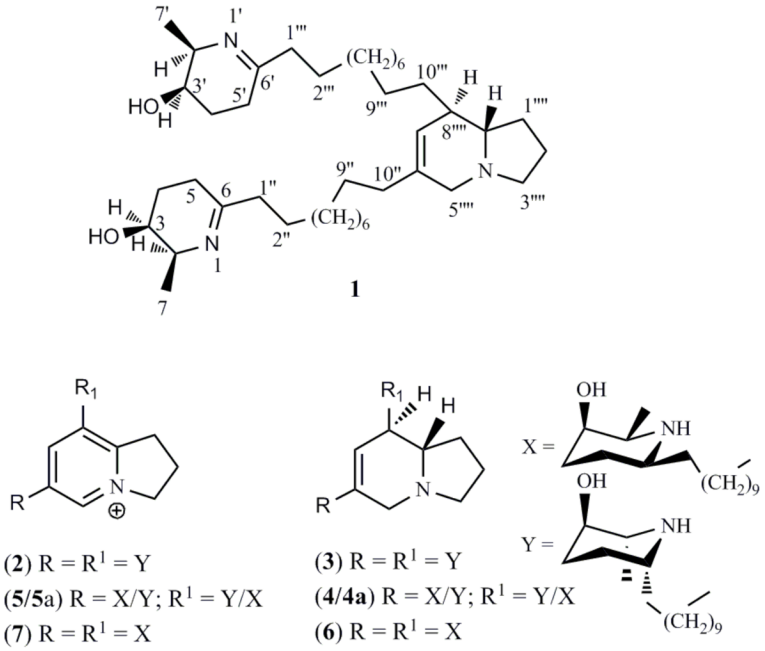

Figure 1.
Compounds (**1**–**7**) isolated from *P. glandulosa.*

Table 1

^{1}H and ^{13}C NMR Data with HMBC correlation (*J* values in Hz, in parenthesis) for **1**.

Position	δ_H	δ_C[a]	HMBC (H-C)	Position	δ_H	δ_C[a]	HMBC (H-C)
2, 2′	3.53 br s	56.1 d	C-4/4′, C-6/6′, C-7/7′	9‴	1.40-1.2[b]	27.1 t	C-6‴′, C-10‴
3, 3′	3.83 br s	66.3 d	C-4/4′, C-5/5′, C-7/7′	10″	1.94 t (7)	35.1 t	C-9″, C-5‴′, C-6‴′, C-7‴′
4, 4′	1.82 m, 1.73m	25.6 t	C-3/3′, C-6/6′	10‴	1.4-1.2[b]	27.9 t	-[b]
5, 5′	2.27m, 2.31m	29.5 t	C-6/6′, C-3/3′, C-1‴	1‴′	1.4-1.15 [b] m	33.1 t	C-3‴′, C-8‴′, C-8a‴′
6, 6′	-	171.1 s	-	2‴′	1.83 m; 1.73 m	21.4 t	C-1‴′, C-8a‴′
7, 7′	1.23[b]	17.5 q	C-2/2′, C-3/3′	3‴′	3.15 m, 2.10 m	54.5 t	C-1‴′, C-5‴′, C-8a‴′
1″, 1‴	2.15 t (7.2)	40.7 t	C-6/6′, C-5/5′	5‴′	3.28 d (15.2) 2.60 d (15.2)	55.2 t	C-3‴′, C-6‴′, C-7‴′, C-8a‴′, C-10″
2″-8″, 2‴-8‴	1.40-1.2 [b]	29.3, 29.4 (x 2), 29.5 (x 2), 29.6, 30.1 (all triplets)	-[b]	6‴′	-	136.0 s	-
				7‴′	5.32 s	123.9 d	C-5‴′, C-8‴′, C-8a‴′, C-10″
				8‴′	1.99 m	42.5 d	C-6‴′, C-7‴′, C-8a‴′
9″		27.1 t	C-10″, C-8‴′	8a‴′	1.77 m	65.5 d	-

Spectra recorded in $CDCl_3$ at 400 MHz (^{1}H) and 100 MHz (^{13}C).

[a] Multiplicities were determined by DEPT and HSQC experiments.

[b] Overlapped signals.

Table 2

Antiplasmodial activities of **1** and **7** and two analogues.

Compound	*P. falciparum* (ng/mL)				VERO (ng/mL)
	D6[a]		W2[b]		IC_{50}
	IC_{50}	SI[c]	IC_{50}	SI[c]	
1	560±5	>43	600±5	>40	>23800
2[d]	39±2	>610	95±2	>250	>23800
6[d]	220±25	23	380±50	13	5000
7	170±35	>140	150±60	>159	>23800
Chloroquine	17		140		>23800
Artemisinin	16		17		>23800

[a] Chloroquine-sensitive clone;

[b] Chloroquine-resistant clone;

[c] Selectivity index= IC_{50} VERO cells/IC_{50} *P. falciparum*;

[d] Data reported previously [8], and used here for comparison with **1** and **7**.

Table 3

Antileishmanial activities of *P. glandulosa* extracts and compounds **1**–**7**.

Extract/Compound	*Leishmania donovani*						Cytotoxicity (Transformed THP1 cells)	
	Promastigotes		Axenic Amastigotes		Macrophage-Amastigotes			
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
P. glandulosa (from Nevada, EtOH extract)	44.2 ± 7.3	91.4 ± 5.2	NT	NT	NT	NT	NT	NT
P. glandulosa (from Texas, EtOH extract)	15.4 ± 2.1	31.9 ± 3.5	NT	NT	NT	NT	NT	NT
P. glandulosa (From Nevada, alkaloid fr.)	18.7 ± 1.9	34.7 ± 2.2	NT	NT	NT	NT	NT	NT
1	0.83 ± 0.11	1.67 ± 0.21	1.83 ± 0.24	5.89 ± 0.76	1.67 ± 0.23	2.48 ± 0.62	8.47 ± 1.32	>20
2	0.91 ± 0.17	4.17 ± 0.54	2.08 ± 0.30	25.4 ± 5.3	3.23 ± 0.54	8.74 ± 1.33	9.49 ± 2.25	>20
3	0.17 ± 0.05	6.33 ± 1.07	0.98 ± 0.12	6.49 ± 0.84	1.08 ± 0.21	1.65 ± 0.22	2.33 ± 0.51	3.89 ± 0.51
5	1.22 ± 0.21	4.52 ± 0.74	2.14 ± 0.32	24.8 ± 3.7	5.87 ± 0.76	9.49 ± 1.14	7.92 ± 1.22	19
6	0.29 ± 0.05	1.14 ± 0.21	1.92 ± 0.19	6.25 ± 0.47	0.79 ± 0.11	1.34 ± 0.24	3.43 ± 0.32	7.89 ± 1.2
7	1.38 ± 0.23	5.85 ± 0.72	2.58 ± 0.37	29.8 ± 4.3	3.08 ± 0.43	7.89 ± 1.91	19.5 ± 1.0	>20
Pentamidine	1.06 ± 0.32	3.86 ± 0.41	2.09 ± 0.37	14.7 ± 2.2	2.97 ± 0.44	6.87 ± 2.11	>20	>20
Amphotericin B	0.09 ± 0.01	0.24 ± 0,04	0.32 ± 0.05	0.97 ± 0.14	0.14 ± 0.02	1.74 ± 0.22	3.34 ± 0.41	8.05 ± 1.33

The results (IC_{50}/IC_{90}) are presented as μg/mL. NT= not tested. Values are mean ± S.D. of three observations.

Table 4

Antimicrobial activity of **1** and **7**.

Compound	IC_{50}/MIC/MFC or MBC (μg/mL)							
	C. albicans	***C. glabrata***	***C. krusei***	***C. neoformans***	***A. fumigatus***	***S. aureus***	**MRSA**	***M. intracellulare***
1	6.7/20/20	-/-/-	5.4/10/10	0.66/1.25/1.25	-/-/-	5.58/10/20	5.36/10/10	9.72/10/20
7	-/-/-	-/-/-	-/-/-	0.17/0.63/0.63	-/-/-	3.0/5.0/10	3.03/5.0/10	0.80/1.25/5
Amphotericin B	0.13/0.63/0.63	0.14/0.63/0.63	0.54/1.25/1.25	0.32/1.25/1.25	0.72/1.25/2.5	NT	NT	NT
Ciprofloxacin	NT	NT	NT	NT	NT	0.1/0.5/-	0.08/0.25/1	0.25/0.5/-

IC_{50} is the concentration that affords 50% inhibition of growth; MIC (minimum inhibitory concentration) is the lowest test concentration that allows no detectable growth; MFC/MBC (minimum fungicidal/bactericidal concentration) is the lowest test concentration that kills the organism; NT = Not tested; (-) = Not active at the highest test concentration of 20 μg/mL.