

Antiparasitic, Nematicidal and Antifouling Constituents from *Juniperus* Berries

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A bioassay-guided fractionation of *Juniperus procera* berries yielded antiparasitic, nematicidal and antifouling constituents, including a wide range of known abietane, pimarane and labdane diterpenes. Among these, abieta-7,13-diene (1) demonstrated *in vitro* antimalarial activity against *Plasmodium falciparum* D6 and W2 strains (IC₅₀ = 1.9 and 2.0 µg/mL, respectively), while totarol (6), ferruginol (7) and 7β-hydroxyabieta-8,13-diene-11,12-dione (8) inhibited *Leishmania donovani* promastigotes with IC₅₀ values of 3.5–4.6 µg/mL. In addition, totarol demonstrated nematicidal and antifouling activities against *Caenorhabditis elegans* and *Artemia salina* at a concentration of 80 µg/mL and 1 µg/mL, respectively. The resinous exudate of *J. virginiana* afforded known antibacterial *E*-communic acid (4) and 4-*epi*-abietic acid (5), while the volatile oil from its trunk wood revealed large quantities of cedrol (9). Using GC/MS, the two known abietanes totarol (6) and ferruginol (7) were identified from the berries of *J. procera*, *J. excelsa* and *J. phoenicea*. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: *Juniperus procera*; *J. excelsa*; *J. phoenicea*; *J. virginiana*; diterpenes; antiparasitic; nematicidal; antifouling.

INTRODUCTION

The genus *Juniperus* (Cupressaceae) has 60–70 species of aromatic evergreen trees or shrubs distributed throughout the Northern Hemisphere, with about 15 species occurring in North America (Adams, 1987; Adams and Turner, 1970). *J. virginiana* Linné (red cedar, red juniper or savin) is a common coniferous species growing on a variety of sites throughout the Eastern half of the United States (Adams, 1987). *J. procera* Hochst ex Endl. (African juniper) is distributed from Tanzania to the Southern part of Saudi Arabia, while *J. excelsa* M.-Bieb. occurs throughout Southeastern Europe, central Asia to the Arabian Peninsula (Collenette, 1985; Kerfoot and Lavranos, 1984). On the other hand, *J. phoenicea* (genévrier Phénicie) obtained in the Mediterranean region, Canary Islands, arid North African Mountains and coastal regions of Yugoslavia. The berry oil from *Juniperus* species is well reputed for a wide spectrum of pharmacological activities and monographs on it are included in various national pharmacopoeias, while *J. procera* is used in the Southern part of Saudi Arabia for the traditional remedy of tuberculosis and jaundice. Earlier investigations on *J. procera* leaves and

stem bark have yielded several antimicrobial diterpenes, including totarol, ferruginol, 4-*epi*-abietic acid, 4-*epi*-abietol, *E*-communic acid and *Z*-communic acid, of which totarol and ferruginol exhibited potentiating activities of INH against four atypical mycobacteria: *M. intracellulare*, *M. smegmatis*, *M. xenopei* and *M. chelonae* (Mossa *et al.*, 1992, 2004; Muhammad *et al.*, 1992, 1995, 1996).

Examinations of the EtOH extract and *n*-hexane partitions of the berries from *J. procera*, *J. excelsa* and *J. phoenicea* showed sufficient antiparasitic activities to warrant further investigation. This led to the identification of previously known abietanes totarol and ferruginol as the principal constituents from these berries, using GC/MS analysis. The EtOH extract of *J. procera* was selected for bioassay-guided fractionation due to its prominent antileishmanial and antimalarial activities. This paper reports the isolation and identification of abieta-7,13-diene (1) as an antimalarial compound, together with the known diterpenes 7α-hydroxyabieta-8,11,13-triene (2), sugiol (3), *E*-communic acid (4), 4-*epi*-abietic acid (5) and sandaracopimeric acid, from *J. procera* berries. In addition, the previously isolated abietane diterpenes totarol (6), ferruginol (7) and 7β-hydroxyabieta-8,13-diene-11,12-dione (8), demonstrated antileishmanial activities, while totarol (6) was identified as the principal constituent exhibiting nematicidal and antifouling activity against *Caenorhabditis elegans* and *Artemia salina*, respectively. The resinous exudate of *J. virginiana*, collected in Texas, afforded the known *E*-communic acid (4) and 4-*epi*-abietic acid (5), while the volatile oil from its trunk wood revealed the presence of large quantities of cedrol (9) and thujopsene, analysed by GC/MS.

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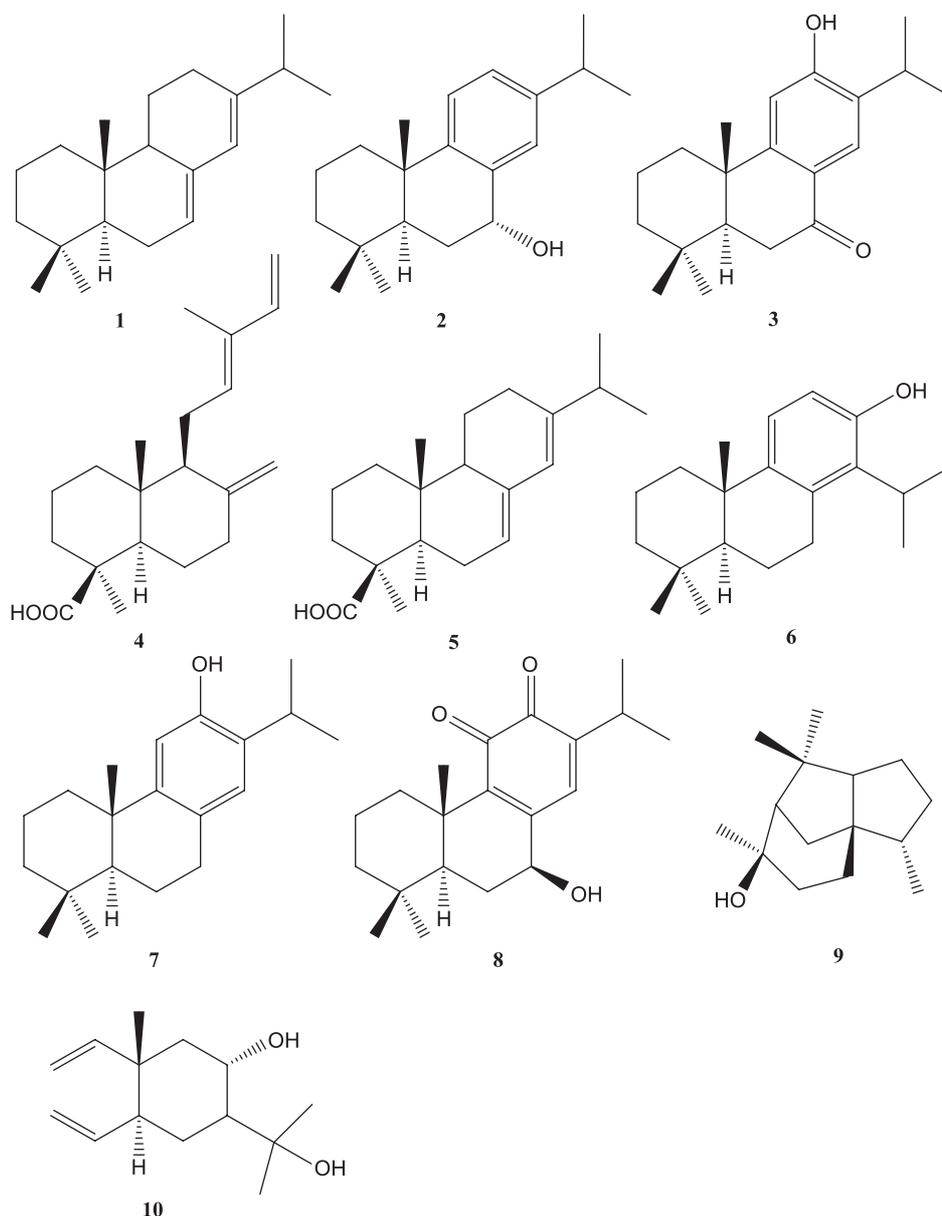
MATERIALS AND METHODS

General. Optical rotations were measured using an AUTOPOL IV[®] instrument at ambient temperature; UV spectra were recorded by a Hewlett Packard 8452A UV/VIS Spectrometer; IR spectra were obtained using Bruker Tensor 27 instrument; ¹H and ¹³C NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 MHz (¹H) and 125 MHz (¹³C), using the residual solvent signal as an internal standard; multiplicity determinations (DEPT 135) and 2D NMR spectra (COSY, NOSEY, HMQC and HMBC) were acquired using standard Bruker pulse programs; HRMS were obtained by direct injection using a Bruker Bioapex-FTMS with Electro-Spray Ionization (ESI) source; TLC was performed on silica gel 60 F-254, with *n*-hexane–EtOAc (9:1) as the solvent system. Spots were visualized by observing under UV-254 nm, followed by spraying with *p*-anisaldehyde spray reagent. Flash column chromatography was performed using silica gel 60, (# 230–400,

ASTM) as adsorbent and *n*-hexane–EtOAc as solvent. Centrifugal preparative TLC (CPTLC; using a Chromatron[®] instrument, Harrison Research Inc. Model 8924) was carried out on a 1 mm Si gel GF coated rotor with F₂₅₄ indicator (Analtech, Inc.), with the solvent system *n*-hexane–EtOAc.

Plant material. The berries of *J. procera*, *J. excelsa* and *J. phoenicea* were collected from Abha, Saudi Arabia, during March 2001. Voucher specimens (14332, 12867 and 14331) were deposited at the herbarium of the College of Pharmacy, King Saud University, Riyadh. The trunk wood and resins of *J. virginiana* were collected in Erath County, TX, in April 2001, and identified by Dr Vaishali Joshi. A voucher specimen (969) was deposited at the herbarium of the School of Pharmacy, University of Mississippi.

Reference standards. Reference standards of 120 different monoterpenes, sesquiterpenes and alkanes used for GC/MS were obtained from commercial sources. The



diterpenes **3-9** and **4-Me** were isolated previously from *J. procera* (Mossa *et al.*, 1992; Muhammad *et al.*, 1992, 1995, 1996).

Extraction of berries and preparation of trunk wood oil. 10 g of the air-dried berries of *J. virginiana*, *J. procera*, *J. excelsa* and *J. phoenicea* were separately and successively extracted at room temperature by maceration with *n*-hexane, followed by EtOH for 24 h (100 mL × 2 times; yields each approximately 0.5 g *n*-hexane extract and 2 g EtOH extract, respectively). Air-dried powdered trunk wood (100 g) of *J. virginiana* was mixed with 500 mL of distilled water and subjected to hydrodistillation for 3 h. The oil obtained was dried over anhydrous Na₂SO₄.

GC/MS analysis. 10 mg of each hexane extract/standard was separately dissolved in 100 µL of chloroform and the sample solutions were filtered using 0.45 µm filters (Millipore, Milford, MA) and 1 µL was injected into GC/MS in triplicate. Thermo Quest Trace MS interfaced to a Thermo Quest Trace GC equipped with an AS 2000 autosampler was used. Column: DB-5 MS (30 m, 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was kept at 60 °C for 2 min and programmed to 240 °C at a rate of 3 °C/min and then kept constant at 240 °C for 3 min; with a total run of 65 min. Helium was used as a carrier gas at a linear velocity 32 cm/s; injection volume 1 µL (10% hexane extract in CHCl₃), split 1:20. The identification of the constituents (Table 1) was carried out using data delivered by the electronic library system, confirmed by comparison of the retention data with those of standards analysed under the same conditions and by comparison with standard mass spectra.

Bioactivity guided isolation of constituents of *J. procera*.

1 kg of the air-dried (crushed) berries of *J. procera* was macerated with 95% EtOH at room temperature for 24 h (1 L each × 3; yield 250 g). The extract (10 g) was fractionated using *n*-hexane/MeCN to afford 7.5 g and 2.0 g, respectively. The *n*-hexane fraction (7 g) was subjected to flash CC over silica gel (600 g), eluted with *n*-hexane and then with *n*-hexane containing increasing amounts of EtOAc (1:0 to 1:1) and the fractions were pooled by TLC. The pooled fractions were purified by CPTLC (1 or 2 mm Si gel GF disc, solvent *n*-hexane–EtOAc (1–10%), flow rate: 3 mL/min) connected to a Fraction Collector. Fr. A (250 mg) was eluted with 3% *n*-hexane–EtOAc, followed by 5% and 10% *n*-hexane–EtOAc. Separation of two bands was monitored under UV light and the collected fractions were dried using a Speed Vac., which afforded **1** (20 mg; [α]_D –5°, *c* 0.8, CHCl₃) and **2** (70 mg; [α]_D –28°, *c* 0.1, CHCl₃; Lit. (Conner *et al.*, 1980) [α]_D –8°, *c* 1.6). Using a similar procedure, fraction C (300 mg) afforded compounds **3** (25 mg) and **4** (25 mg), and fraction D (200 mg) yielded **5** (50 mg). The identity of compounds **1** and **2** were established by comparison with physical (mp and OR) and spectral data (UV, IR, NMR and MS) with those published (De Pascual Teresa *et al.*, 1975, 1978), and **3–5** were confirmed by comparison with reference authentic samples, as well as their physical and spectral data (Mossa *et al.*, 1992; Muhammad *et al.*, 1992, 1995, 1996).

Isolation of constituents from *J. virginiana* resin. Dried colorless resin droplets (1.5 g) were scraped from the inner bark of *J. virginiana*. They were cleaned from dirt and fibers, and dissolved in CH₂Cl₂ and filtered through celite and dried (1.3 g). A sample of 500 mg was subjected to centrifugal preparative TLC (Chromatotron®),

Table 1. Antileishmanial and antimalarial activity of *Juniperus* berry extracts and isolated compounds

Extract/Compound	<i>L. donovani</i>			<i>P. falciparum</i> W2	Vero TC ₅₀ (µg/mL)
	IC ₅₀	IC ₉₀	D6		
	(µg/mL)			IC ₅₀ (µg/mL)	
<i>J. procera</i> EtOH extract	9.6	30	1.5 (>31.7)	2.9 (>16.4)	NC
<i>J. procera</i> hexane Fr.	4.7	11	5.8 (6.7)	4.4 (8.9)	39
<i>J. procera</i> MeCN Fr.	18	50	NA	NA	NC
<i>J. excelsa</i> hexane Fr.	NA	NA	5.8 (>8.2)	3.3 (>14.4)	NC
<i>J. phoenicea</i> EtOH extract	38	50	5.0 (>9.5)	2.8 (>17)	NC
<i>J. phoenicea</i> hexane Fr.	25	43	NA	NA	NC
Totarol (6)	3.5	6.9	NA	NA	NC
Ferruginol (7)	3.5	7.0	4.2 (>1.1)	3.5 (>1.4)	NC
7β-hydroxyabieta-8,13-diene-11,12-dione (8)	4.6	22	NA	NA	NC
Abieta-7,13-diene (1)	17	34	1.9 (2.5)	2.0 (>2.4)	NC
Hinokiol	18	35	NA	NA	NC
Hinokiol-1-one	18	35	NA	NA	NC
4-Epi-abietol	34	>40	NA	NA	NC
Abieta-8,11,13-trien-7α-ol (2)	17	34	NA	NA	NC
Sugiol (3)	–	–	3.0 (>1.6)	>4.7 (>1.0)	NC
Cedrol (9)	15	33	4.4 (>1.1)	>4.7 (>1.0)	>4.7
Sandaracopimeric acid	23	>40	NA	NA	NC
Z-Communic acid					
Me ester (4-Me)	19	40	NA	NA	NC
Pentamidine	1.3	7.0	NT	NT	NT
Chloroquine	NT	NT	<0.264	0.15	NT

+, active; NA, no activity; NC, no cytotoxicity at 4.76 µg/mL; NT, not tested; D6, chloroquine-sensitive clone. W2, chloroquine-resistant clone; Selectivity index (in parentheses) = IC₅₀ Vero cells/IC₅₀ *P. falciparum*.

4 mm silica gel disc), using *n*-hexane–EtOAc as eluant, which afforded *E*-communic acid (**4**) as amorphous solid {425 mg, $[\alpha]_D^{25} +39.5^\circ$ (*c* 1.50, CHCl₃); Lit. (Lee *et al.*, 1987) $[\alpha]_D^{25} +38.1^\circ$ }, followed by **5** as granules {35 mg, mp 155–157°, $[\alpha]_D^{25} -72.5^\circ$ (*c* 1.50, CHCl₃); Lit. (Buckingham, 1982) mp 156–157°, $[\alpha]_D^{25} -74^\circ$ (*c* 1.50, CHCl₃)}. The identity of compounds **4** and **5** was confirmed by comparison with reference samples of *E*-communic acid and 4-*epi*-abiatic acid, respectively.

Antimalarial assay. The *in vitro* antimalarial activity was determined against two strains of *P. falciparum* [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. The assay (Makler *et al.*, 1993) is based on the determination of plasmodial LDH activity using Malstat™ reagent and is performed in 96-well plates. The extracts were first screened in a primary assay at one concentration (15.9 µg/mL) and those exhibiting an inhibition of parasite growth by 50% or more were further tested at three concentrations (47.6, 15.9, 5.3 µg/mL) to obtain IC₅₀ values. Pure compounds were screened at six concentrations of 3-fold dilutions (4.76–0.195 µg/mL) to obtain IC₅₀ values. The level of toxicity of each sample is also determined towards the mammalian kidney fibroblast cell line (Vero) (Babich and Borenfreund, 1991) and TC₅₀ to Vero cell is determined to calculate the selectivity index (SI). The standard antimalarial agent chloroquine is used as a positive control and DMSO is used as a vehicle control.

Antileishmanial assay. Activity of the extracts and compounds against a culture of *L. donovani* promastigotes was tested *in vitro*. The promastigotes were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco Chemical Co.) at 26 °C. A 3-day-old culture was diluted to 5 × 10⁵ promastigotes/mL. Drug dilutions (50–3.1 µg/mL) were prepared directly in cell suspension in 96-well plates. Plates were incubated at 26 °C for 48 h, and the growth of leishmanial promastigotes was determined by the Alamar blue assay (Mikus and Steverling, 2000). Standard fluorescence was measured by a Fluostar Galaxy plate reader (BMG LabTechnologies) at an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Pentamidine and amphotericin B were used as the control drugs. The percent growth

was calculated and plotted with the concentration tested for computing the IC₅₀ and IC₉₀ values.

Bactericidal activity. To each well of a 96-well microtiter plate, 200 µL tryptose agar (20 g bacto tryptose agar, 5 g sodium chloride, 1 g bacto dextrose and 15 g agar in 1 L deionized water), containing the test compounds at a concentration of 100 µg/mL (Table 3), were added. The nutrient medium was inoculated by adding 2 µL of an actively growing liquid culture of *P. aeruginosa* in tryptose broth (without agar), and incubated in the dark at 27 °C with 70% relative humidity. The growth was evaluated after 5 days.

Fungicidal activity. To each well of a 96-well microtiter plate, 200 µL potato dextrose agar (PDA, 4 g potato infusion, 20 g bacto dextrose and 15 g bacto agar in 1 L deionized water), containing the test compounds at a concentration of 10 mg/mL (Table 3), were added. The nutrient medium was inoculated with *Aureobasidium pullulans*, *Aspergillus niger*, *Chaetomium globosum*, *Colletotrichum musae*, *Phytophthora citricola* or *Septoria nodorum* by adding a spore/mycelium suspension (2 µL) or a small piece of agar from the margin of an actively growing colony, and incubated in the dark at 27 °C with 70% relative humidity. The growth of the fungi was evaluated after 1 week.

Algicidal activity. To each well of a 96-well microtiter plate, 270 µL BG11 medium (Stanier *et al.*, 1971: 1.5 g NaNO₃, 40 mg K₂HPO₄, 75 mg MgSO₄·7H₂O, 36 mg CaCl₂·2H₂O, 6 mg citric acid, 6 mg ammonium ferric citrate green, 1 mg EDTANa₂, 20 mg Na₂CO₃, 2.86 mg H₃BO₃, 1.81 mg MnCl₂·4H₂O, 0.22 mg ZnSO₄·7H₂O, 0.39 mg Na₂MoO₄·2H₂O, 0.08 mg CuSO₄·5H₂O, 0.05 mg Co(NO₃)₂·6H₂O in 1 L deionized water), containing the test compounds at a concentration of 1 µg/mL (Table 3), were added. The liquid freshwater mineral medium was inoculated by adding 30 µL of an actively growing, 3 week old liquid BG11 culture of *Chlorella vulgaris* and incubated at 21 °C, 65% relative humidity, 1000 Lux 16 h/day. The growth was evaluated after 2 weeks.

Antifouling activity. *Artemia salina* was used as a model organism for crustacean marine foulers, such as

Table 2. Activities against plant pathogens and biodeterioration organisms of *Juniperus* extract and isolated compounds

Extract/Compound	Field/Organism		
	Antifouling: <i>Artemia salina</i>	Nematicide: <i>Caenorhabditis elegans</i>	Fungicide: (An, Ap, Cg, Cm, Pc, Sn) ^a
<i>J. procera</i> (EtOH ext.)	+++	+	+
<i>J. excelsa</i> (EtOH ext.)	+++	+	+
<i>J. phoenicea</i> (EtOH ext.)	+	+	++
<i>J. virginiana</i> leaves (EtOH ext.)	+	++	+
<i>J. virginiana</i> root (EtOH ext.)	+	+++	+
Totarol (6)	+++	+++	+
Ferruginol (7)	+	++	+
8α-Hydroxyelemol (10)	++	+	+
Control ^b	+++	+++	+++

+, weakly active; ++, moderately active; +++, strongly active; Test concentrations of pure compounds for: *Pseudomonas aeruginosa*, 100 µg/mL; *Caenorhabditis elegans*, 80 µg/mL; An, Ap, Cg, Cm, Pc, Sn*, 10 µg/mL; *Chlorella vulgaris*, *Artemia salina*, 1 µg/mL. Extracts were tested at ten-fold higher concentrations.

^a An, *Aspergillus niger*; Ap, *Aureobasidium pullulans*; Cg, *Chaetomium globosum*; Cm, *Colletotrichum musae*; Pc, *Phytophthora citricola*; Sn, *Septoria nodorum*.

^b Jansen Pharmaceutica reference compounds.

barnacles. *Artemia salina* cysts (Great Salt Lake strain) were allowed to hatch in aerated artificial seawater according to Probst, with the following composition (mg/L): NaCl, 27187; MgSO₄·7H₂O, 6931; MgCl₂·6H₂O, 5112; CaCl₂·2H₂O, 1529; KCl, 681; KBr, 98; KF, 4; KI, 0.06; NaHCO₃, 200; Na₂CO₃·10H₂O, 111; H₃BO₃, 28; Sr(NO₃)₂, 15; C₆H₅Li₃O₇·4H₂O (lithium citrate), 2.8; PVP (polyvinylpyrrolidone), 2.5; C₆H₅FeO₇·5H₂O (iron citrate), 0.3; RbCl, 0.3. Approximately 30 *A. salina* instar II larvae obtained in this way were added to 2 mL of the same artificial seawater, containing the test compounds at a concentration of 1 mg/L (Table 3), in each well of a 24-well microtiter plate. The organisms were incubated in the laboratory at 25 °C and survival of the crustaceans was evaluated after 1 day (He *et al.*, 2001, 2002).

Nematicidal activity. To each well of a 96-well microtiter plate, 100 µL of liquid nematode growth medium (NGM, Riddle *et al.*, 1997: 3 g NaCl, 2.5 g bacto-peptone, 5 mg cholesterol, 3.4 g K₂HPO₄, 448 mg KOH, 111 mg CaCl₂, 120 mg MgSO₄ in 1 L deionized water) containing 20 *Caenorhabditis elegans* larvae and *Escherichia coli* as a food source was added. Test compounds were dissolved in 0.5 µL DMSO and added to the NGM medium to a final concentration of 80 µg/mL (Table 3). The animals were incubated in the laboratory at 21 °C and 65% relative humidity. Survival of the nematodes was evaluated after 1 day.

Insecticidal activity. In the wells of a 24-well microtiter plate, 1 g of wheat grains was treated with an appropriate amount of test compound, dissolved in 1 mL MeOH, in order to reach a final dose of 100 mg/kg seed. The solvent was evaporated on a hot plate at 55 °C and subsequently 30 adult *Sitophilus granarius* were added, and incubated at 23 °C with 70% relative humidity. The evaluation of insect survival was done after 1 week and development of second generation was evaluated after 7 weeks.

RESULTS AND DISCUSSION

The EtOH extracts of *J. procera* and *J. phoenicea* berries showed antimalarial and antileishmanial activities (Table 2), while *J. excelsa* and *J. procera* demonstrated strong antifouling activities against *Artemia salina*. In addition, upon solvent fractionation the antimalarial and antileishmanial activities of *J. procera* resided in hexane fraction with IC₅₀ values 4.7, 5.8 and 4.4 µg/mL against *P. falciparum* D6 and W2 clones, and *L. donovani*, respectively. Column chromatography followed by centrifugal preparative thin layer chromatography (CPTLC) of the *n*-hexane fraction resulted in the isolation of abieta-7,13-diene (**1**) (De Pascual Teresa *et al.*, 1978), 7 α -hydroxyabieta-8,11,13-triene (**2**) (De Pascual Teresa *et al.*, 1975), sugiol (**3**), *E*-communic acid (**4**) and 4-*epi*-abietic acid (**5**) (Muhammad *et al.*, 1992, 1995, 1996). The presence of totarol (**6**) and ferruginol (**7**) in the *n*-hexane partition was identified by GC/MS. These compounds were available from our earlier isolation work on *J. procera*. The structures of isolated compounds were established by ¹H and ¹³C NMR data, including 2D NMR COSY, HMQC, HMBC and NOESY experiments, and HRMS. In addition, a total of 21 diterpenes and sesquiterpenes, including 7 β -hydroxyabieta-8,13-diene-

11,12-dione (**8**) and 8 α -hydroxyelemol (**10**), were isolated previously from the leaves and stem bark of *J. procera*.

Antiparasitic activities

Significant antileishmanial and antimalarial activities were observed in the berries of African juniper, *J. procera*. The activity of the *n*-hexane partition of *J. procera* EtOH extract was considered to be prominent among all the fractions investigated (Table 2). Assay guided fractionation of the *n*-hexane partition yielded diterpenes **1–5**, of which abieta-7,13-diene (**1**) demonstrated moderate antimalarial activity against *P. falciparum* D6 and W2 clones with IC₅₀ values of 1.9 and 2.0 µg/mL, respectively, while the corresponding activity of the crude *n*-hexane fraction was 5.8 and 4.4 µg/mL. The enhanced antimalarial activity of *J. procera* *n*-hexane fraction might be due to the presence of additional more potent antimalarial constituents, but it might also be due to synergism with other components in the extract. On the other hand, totarol (**6**), ferruginol (**7**) and 7 β -hydroxyabieta-8,13-diene-11,12-dione (**8**) showed good antileishmanial activity (IC₅₀ values of 3.5–4.6 µg/mL vs 1.3 µg/mL for pentamidine) against *L. donovani* promastigotes.

Activities against plant pathogens and biodeterioration organisms

The EtOH extracts of *J. procera*, *J. excelsa*, *J. phoenicea* and *J. virginiana*, and isolated compounds from the berry, leaf and stem bark of *J. procera*, including compounds **1–10**, were tested in a primary screen against 12 test organisms at a single concentration for each observation. The primary assays covered six fields of interest, namely bactericide, fungicide, algicide, nematicide, antifouling and insecticide activities. An appropriate reference compound of Janssen Pharmaceutica was used as a positive control in each of these fields. Totarol (**6**) showed strong nematicide activity at a concentration of 80 µg/mL, as well as activity against the crustacean animal *Artemia salina*, a model organism for crustacean foulers like barnacles, at 1 µg/mL (Table 3). The sesquiterpene 8 α -hydroxyelemol (**10**) demonstrated moderate activity against *Artemia salina*, while ferruginol (**7**) only showed moderate nematicidal activity against *Caenorhabditis elegans*. As far as the plant extracts are concerned, the *J. procera* and *J. excelsa* extracts showed activity against *Artemia salina*, suggesting possible antifouling activity, while the leaves and root of *J. virginiana* showed nematicidal activity. All the extracts and compounds tested failed to show bactericidal, algicidal and insecticidal activities.

Difference in constituents between *J. virginiana* and *J. procera* resins

The resinous exudates of *J. virginiana* yielded two known diterpenes, namely *E*-communic acid (**4**) and 4-*epi*-abietic acid (**5**), as the main constituents in yields of ~85% and ~7%, respectively. Its antibacterial activity resided on the diterpenes **4** and **5**, previously isolated from *J. procera* (Mossa *et al.*, 1992; Muhammad *et al.*, 1992, 1995, 1996).

Table 3. GC/MS analysis of the *n*-hexane extract of the berries of *J. procera*, *J. excelsa*, *J. phoenicea* and essential oil of *J. virginiana* berries and trunk wood oil

Compound	R _t	M ⁺	Area (in %)				
			A	B	C	D	E
α -Pinene	5.60	136	15.2	7.1	22.9	0.4	1.6
α -Phellandrene	6.76	136	–	–	–	1.1	–
β -Myrcene	7.32	136	0.5	–	0.4	0.7	–
3-Carene	8.00	136	8.9	4.3	–	–	–
D-Limonene	8.80	136	–	–	–	59.3	–
β -Phellandrene	8.82	136	0.5	–	–	–	–
α -Cedrin	25.76	204	–	–	–	–	0.6
β -Caryophyllene	25.94	204	3.7	1.5	1.0	–	0.2
β -Cedrin	26.10	204	–	–	–	–	0.7
Thujopsene	26.71	204	–	–	–	–	31.2
α -Caryophyllene	27.50	204	4.5	1.5	0.8	–	–
Germacrene-D	28.61	204	2.1	0.9	2.0	–	0.6
Unknown	31.52	204	–	–	–	16.7	–
Cedrol (9)	34.01	222	–	–	–	–	42.8
Cubenol	34.68	204	–	–	1.0	0.9	4.8
α -Bisabolol	35.70	204	–	–	–	6.4	2.4
Unknown	50.95	300	5.0	6.0	0.5	–	–
Unknown	53.97	302	0.8	1.2	1.2	–	–
17 α -Hydroxyandrost-4-en-3-one	54.93	288	–	1.6	–	–	–
4- <i>epi</i> -Abietal	56.56	286	–	–	23.9	–	–
Totarol (6)	57.69	286	1.2	1.2	1.5	–	–
Ferruginol (7)	58.22	286	7.3	32.9	0.7	–	–
Hinokiol-1-one	60.33	316	10.6	5.9	4.3	–	–
Unknown	60.85	316	7.4	1.3	6.0	–	–
Hinokiol	63.94	302	0.7	0.6	0.9	–	–

R_t, retention time in minutes; M⁺, molecular ion; A, *J. procera* berries; B, *J. excelsa* berries; C, *J. phoenicea* berries; D, *J. virginiana* berries; E, *J. virginiana* trunk wood.

In contrast, *E*- and *Z*-communic acids are the main constituents isolated from the resinous exudates of *J. procera*. These isomeric constituents (yields 45% and 55%, respectively) constitute about 90% of the total weight in *J. procera* resin.

GC/MS analysis

GC/MS analysis (Table 1) of the *J. procera* hexane fraction revealed significant amounts of ferruginol (7) (7.3%), hinokiol-1-one (10.6%) and α -pinene (15.2%). In contrast, *J. excelsa* demonstrated 32.9% ferruginol, while *J. phoenicea* contained 23.9% of 4-*epi*-abietal. The antibacterial activity of these berry extracts is due to the presence of terpenoids, especially the well-known diterpenes totarol (6) and ferruginol (7). GC/MS analysis of the berry oil and cedar oil (trunk wood oil) of *J. virginiana* revealed significantly different compositions with those observed for the other *Juniperus* berries. The berry oil contains about 59.3% D-limonene, while cedrol (8) and thujopsene (9) are the major components of the cedar oil, obtained in the yields of 42.8% and 31.2%, respectively. The EtOH extracts of the berries and trunk wood of *J. virginiana* were found to be inactive in antiparasitic assays.

CONCLUSION

This appears to be the first report of antimalarial activity of abietadine (1), and antileishmanial, nematicidal

and antifouling activity of totarol (6), isolated from *J. procera*. However, two abietatrienes (Clarkson *et al.*, 2003), dehydroabietinol (Ziegler *et al.*, 2002), abietane ester and benzylsloppone (Achenbach *et al.*, 1992) have shown antimalarial activity against *P. falciparum* previously. A significant difference in the antimalarial and antileishmanial activities was observed between the berries of *J. virginiana* and other junipers (*J. procera*, *J. excelsa* and *J. phoenicea*). The activity of *J. procera* is considered to be most prominent among the entire juniper berries investigated. The quantitative yield of the diterpenes from *J. procera*, *J. excelsa* and *J. phoenicea* were also found to be significantly different. Thus, the content of ferruginol (7) was significantly high in *J. excelsa*, while 4-*epi*-abietal was only present in *J. phoenicea*. A clear difference in mono- and sesquiterpene composition was noted between the berries of native juniper (*J. virginiana*) with other three species. Thus, D-limonene was found to be the major constituent of *J. virginiana*, while high amounts of α -pinene was present in *J. procera* and *J. phoenicea*. In addition, the composition of *J. virginiana* trunk wood oil was found to be significantly different compared with its berry oil. The remarkably high yield of *E*-communic acid (4) from the native *Juniperus* resin could be utilized as a commercially viable source of 4.

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