SUPPLEMENTARY MATERIAL

Antileishmanial diterpenoid alkaloids from *Aconitum spicatum* (Bruhl) Stapf

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The crude extracts of tubers of *Aconitum spicatum* (Bruhl) Stapf were investigated for *in vitro* antileishmanial activity against *Leishmania major*. The dichloromethane extract at pH 2.5 showed antileishmanial activity with IC₅₀ value of 27.10 ± 0.0 µg/mL.

Chromatographic purification of the dichloromethane extract led to isolation of three C-19 norditerpenoid alkaloids indaconitine (**1**), chasmaconitine (**2**) and ludaconitine (**3**).

Compounds **3** and **2** showed antileishmanial activity with IC₅₀ = 36.10 ± 3.4 and 56.30 ± 2.1 µg/mL, respectively. Compound **1** was less effective (IC₅₀ >100 µg/mL). The cytotoxicity of compounds **1**, **2** and **3** studied against MCF7, HeLa and PC3 cancer cell lines and 3T3 normal fibroblast cell line did not show cytotoxicity at 30 µM.

Keywords: *Aconitum spicatum*, antileishmanial activity, *Leishmania major*, diterpenoid alkaloids

Experimental

Collection of plant material

The tubers of *A. spicatum* were collected from Panch Pokhari, Sindhupalchok district of central Nepal, at the altitude of 3800 m. The specimen of the plant was identified by comparing the

herbarium (voucher no.59185) deposited at National Herbarium Laboratory, Godawari, Lalitpur, Nepal.

Chemicals

Commercially available analytical grade solvents were used for solvent-solvent extraction and column chromatography. HPLC grade solvents were purchased from Merck and Fisher Scientific companies. Column chromatography was performed by using silica gel (Merck, 200-400 mesh). Pre-coated silica gel plates (Merck, silica gel 60 F₂₅₄) were used for thin layer chromatography (TLC).

Extraction and chromatographic separation of compounds

The tubers were chopped into small pieces, and dried under shade. The dried tuber (6.0 Kg) was grinded into the fine powder, and was extracted thrice with 8 L of 1% HCl in methanol:water (85:15) for 72 h. The viscous dark red residue (1.2 kg) was obtained after concentration under reduced pressure. The crude extract (500 g) suspended in water, was extracted by solvent-solvent extraction to obtain *n*-hexane (4.2 g); acidic (prepared by adding CH₃COOH) and basic dichloromethane (prepared by adding NH₄OH) at pH 2.5 (14.0 g), pH 4 (0.16 g), pH 7 (3.2 g) and pH 11 (0.2 g); ethyl acetate (1.0 g) and *n*-butanol (21.6 g) extracts. The acidic dichloromethane extract at pH 2.5 (14.0 g) was column chromatographed on silica gel and eluted with *n*-hexane. Then polarity was gradually increased by adding ethyl acetate, followed by methanol. Two major extracts were compiled. The extract A was compiled from less polar eluents by increasing percentage of ethyl acetate in *n*-hexane. The extract B was compiled from polar eluents when percentage of methanol in ethyl acetate was gradually increased. The extract B was loaded onto silica gel column and eluted with dichloromethane/methanol to isolate

indaconitine (**1**), chasmaconitine (**2**) and ludaconitine (**3**). The extract A was repeatedly run into normal phase recycling HPLC (LC 908 column) eluting with ethyl acetate:*n*-hexane (20:80) to get emodin (**4**), *trans*-methyl-*p*-coumarate (**5**), *p*-hydroxybenzaldehyde (**6**) and ursolic acid (**7**). The dichloromethane extract at pH 4 (0.16 g) was chromatographed in silica gel column using *n*-hexane/ethyl acetate solvent system to obtain 5-(hydroxymethyl)-furan-2-carbaldehyde (**8**). β -Sitosterol glucoside (**9**) was obtained from dichloromethane extract at pH 11 (0.2 g) by silica gel column chromatography eluting with dichloromethane/methanol. The structures were identified by comparing their spectroscopic data with the literature. The modern spectroscopic techniques, such as UV, IR, ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC, EI, FAB and ESI MS were used for structure determination.

Characterization of compounds

Indaconitine (1) [CAS No. 4491-19-4]

Indaconitine (**1**)

White amorphous (100 mg); melting point 197-198 °C; EI MS m/z : 629 $[\text{M}]^+$; molecular formula: $\text{C}_{34}\text{H}_{47}\text{NO}_{10}$; ^1H NMR (500 MHz, CDCl_3) δ ppm: 3.11 (*m*, H-1), 2.03 (*m*, H-2), 2.33 (*m*, H-2), 3.73 (*dd*, $J = 9.5, 5$ Hz, H-3), 2.06 (*s*, H-5), 4.00 (*d*, $J = 6.5$ Hz, H-6), 3.02 (H-7), 2.89 (H-9), 2.07 (H-10), 2.06 (H-12), 2.60 (H-12), 4.88 (*d*, $J = 5$ Hz, H-14), 2.40 (H-15), 2.97 (H-15), 3.37 (*dd*, $J = 8.5, 6.5$ Hz, H-16), 2.99 (H-17), 3.50 (*d*, $J = 9$ Hz, H-18), 3.62 (*d*, $J = 9$ Hz, H-18),

2.33 (H-19), 2.89 (H-19), 2.44 (*m*, H-20), 2.53 (H-20), 1.07 (*t*, $J = 7$ Hz, H-21), 3.23 (*s*, OMe-1), 3.13 (*s*, OMe-6), 3.52 (*s*, OMe-16), 3.27 (*s*, OMe-18), 1.26 (*s*, COCH₃-8), 8.03 (*d*, $J = 7$ Hz, H-2' and H-6'), 7.42 (H-3' and H-5'), 7.54 (H-4'); ¹³C-NMR (125 MHz, CDCl₃) δ ppm: 82.2 (C-1), 33.5 (C-2), 71.6 (C-3), 43.1 (C-4), 40.8 (C-5), 83.1 (C-6), 48.7 (C-7), 85.5 (C-8), 44.7 (C-9), 47.4 (C-10), 50.2 (C-11), 35.2 (C-12), 74.7 (C-13), 78.8 (C-14), 39.5 (C-15), 83.5 (C-16), 61.6 (C-17), 77.0 (C-18), 47.4 (C-19), 48.8 (C-20), 13.3 (C-21), 55.8 (OMe-1), 57.7 (OMe-6), 58.7 (OMe-16), 59.1 (OMe-18), 169.8 (COCH₃-8), 21.5 (COCH₃-8), 166.2 (CO-14), 130.1 (C-1'), 129.6 (C-2' and C-6'), 128.5 (C-3' and C-5'), 133.1 (C-4') (Khetwal et al. 1994).

EI Mass spectrum

ICCBS-HEJ[LAB-104]
1/21/2014

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IR spectrum

¹H NMR (500 MHz, CDCl₃)

¹³C-NMR (125 MHz, CDCl₃)

Chasmaconitine (2) [CAS No. 6846-46-4]

Chasmaconitine (2)

White crystalline (300 mg); melting point 184 °C, EI MS m/z : 613 $[M]^+$ molecular formula: C₃₄H₄₇NO₉; ¹H NMR (500 MHz, CDCl₃) δ ppm: 3.10 (*m*, H-1), 1.94 (*m*, H-2), 2.25 (*m*, H-2), 1.63 (*m*, H-3), 2.14 (*s*, H-5), 3.98 (*d*, J = 6.5 Hz, H-6), 2.99 (H-7), 2.90 (H-9), 2.16 (H-10), 2.05

(H-12), 2.71 (H-12), 4.89 (*d*, $J = 5$ Hz, H-14), 2.45 (H-15), 2.94 (H-15), 3.38 (*dd*, $J = 9, 6$ Hz, H-16), 2.96 (H-17), 3.20 (*d*, $J = 8.5$ Hz, H-18), 3.61 (*d*, $J = 8.5$ Hz, H-18), 2.48 (*m*, H-19), 2.52 (*m*, H-19), 2.43 (*m*, H-20), 2.57 (*m*, H-20), 1.10 (*t*, $J = 6$ Hz, H-21), 3.52 (*s*, OMe-1), 3.15 (*s*, OMe-6), 3.27 (*s*, OMe-16), 3.26 (*s*, OMe-18), 1.22 (*s*, COCH₃-8), 8.09 (*d*, $J = 7$ Hz, H-2' and H-6'), 7.48 (*m*, H-3' and H-5'), 7.60 (*m*, H-4'); ¹³C-NMR (125 MHz, CDCl₃) δ ppm: 85.8 (C-1), 27.0 (C-2), 35.5 (C-3), 40.0 (C-4), 50.1 (C-5), 86.8 (C-6), 50.1 (C-7), 86.8 (C-8), 46.3 (C-9), 42.0 (C-10), 51.3 (C-11), 37.5 (C-12), 76.2 (C-13), 80.2 (C-14), 40.7 (C-15), 85.0 (C-16), 62.8 (C-17), 81.3 (C-18), 54.9 (C-19), 50.0 (C-20), 13.6 (C-21), 59.4 (OMe-1), 58.4 (OMe-6), 56.2 (OMe-16), 59.4 (OMe-18), 171.3 (COCH₃-8), 21.7 (COCH₃-8), 167.7 (CO-14), 131.3 (C-1'), 130.7 (C-2' and C-6'), 129.6 (C-5' and C-3'), 134.3 (C-4') (Yue et al. 1990).

EI Mass spectrum

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File: 3C-DF-II Date Run: 01-20-2014 (Time Run: 16:06:04)
Sample: TENSANGMU L. TAMANG/ DR. M. IQBAL
Instrument: JEOL JMS 600-H
Inlet: My Inlet Ionization mode: EI+

IR spectrum

3C-DF-ILIRS: 3C-DF-II/Tensangmu/MIC
 Date: 04/08/2014 Time: 09:49:28 NScans: 10
 Type: HYPER IR User: ZUBAIR AHMED Detector: standard
 Abscissa: 1/cm Ordinate: %T Apodization: Happ
 Min: 400.20 Max: 3999.12 Range: 1/cm
 Ndp: 3733 Data Interval: 0.96434 Resolution: 2.0

¹H NMR (500 MHz, CDCl₃)

TENSANGMU L.TAMANG/DR.IQBAL/3C-DF-II/CD3OD+CDCL3

AVANCE AV-500
LAB NO: 109B

NAME jan21-14
EXPNO 7
PROCNO 1
Date_ 20140121
Time 14.56
INSTRUM spect
PROBHD 5 mm BBI 1H/D-
PULPROG zg30
TD 32768
SOLVENT MeOD
NS 64
DS 0
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860696 sec
RG 71.8
DW 48.400 usec
DE 6.50 usec
TE 300.0 K
D1 2.00000000 sec
TD0 1
===== CHANNEL f1 =====
NUC1 1H
P1 8.00 usec
PL1 -1.00 dB
SFO1 500.1335009 MHz
SI 32768
SF 500.1300158 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

^{13}C -NMR (125 MHz, CDCl_3)

Ludaconitine (3)

Ludaconitine (3)

White crystalline (18 mg); EI MS m/z : 587 $[\text{M}]^+$; molecular formula: $\text{C}_{32}\text{H}_{45}\text{NO}_9$; ^1H NMR (500 MHz, CDCl_3) δ ppm: 3.60 (*m*, H-1), 2.26 (*m*, H-2), 2.29 (*m*, H-2), 4.18 (*m*, H-3), 2.46 (*s*, H-5), 4.22 (*d*, $J = 6.5$ Hz, H-6), 2.18 (H-7), 2.61 (H-9), 2.42 (H-10), 5.01 (*d*, $J = 5.5$ Hz, H-14), 2.47 (H-15), 2.54 (H-15), 3.48 (H-16), 3.31 (H-17), 3.51 (H-18), 3.52 (H-18), 3.58 (H-19), 3.30 (H-

20), 1.39 (*t*, $J = 7.5$ Hz, H-21), 3.39 (*s*, OMe-1), 3.35 (*s*, OMe-6), 3.55 (*s*, OMe-16), 3.31 (*s*, OMe-18), 8.10 (*d*, $J = 7$ Hz, H-2' and H-6'), 7.59 (H-3' and H-5'), 7.46 (H-4'); ^{13}C -NMR (125 MHz, CDCl_3) δ ppm: 81.3 (C-1), 30.1 (C-2), 70.6 (C-3), 43.8 (C-4), 44.3 (C-5), 83.0 (C-6), 56.1 (C-7), 74.6 (C-8), 45.7 (C-9), 41.5 (C-10), 52.1 (C-11), 36.7 (C-12), 76.4 (C-13), 80.5 (C-14), 43.8 (C-15), 84.1 (C-16), 66.9 (C-17), 78.1 (C-18), 51.0 (C-19), 50.7 (C-20), 11.0 (C-21), 55.3 (OMe-1), 58.5 (OMe-6), 59.4 (OMe-16), 59.4 (OMe-18), 167.8 (CO-14), 131.6 (C-1'), 131.0 (C-2' and C-6'), 129.4 (C-3' and C-5'), 134.2 (C-4') (Khetwal et al. 1992).

EI Mass spectrum

ICCBS-HEJ/LAB-104/
1/21/2014

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File: DM-8-59 Date Run: 01-20-2014 (Time Run: 16:13:36)
Sample: TENSANGMU L. TAMANG/ DR. M. IQBAL
Instrument: JEOL JMS 600-H
Inlet: My Inlet Ionization mode: EI+

IR spectrum

¹H NMR (500 MHz, CDCl₃)

TENSANGMU L.TAMANG/DR.IQBAL/DM8-59/CD3OD

AVANCE AV-500
LAB NO: 109B

NAME jan10-14
EXPNO 7
PROCNO 1
Date_ 20140110
Time_ 15.39
INSTRUM spect
PROBHD 5 mm BBI 1H/D-
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 128
DS 0
SWH 10330.378 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 362
DW 48.400 usec
DE 6.50 usec
TE 300.0 K
D1 1.50000000 sec
TD0 1

===== CHANNEL f1 =====
NUC1 1H
P1 8.00 usec
PL1 -1.00 dB
SFO1 500.1335009 MHz
SI 32768
SF 500.1300158 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

^{13}C -NMR (125 MHz, CDCl_3)

Emodin (**4**) [CAS No 518-82-1]

Emodin (**4**)

Orange crystalline (3 mg); melting point: 260 °C; EI MS m/z : 270 $[\text{M}]^+$; molecular formula:

$\text{C}_{15}\text{H}_{10}\text{O}_5$; ^1H NMR (300 MHz, $\text{C}_3\text{D}_6\text{O}$) δ ppm: 6.66 (d , $J = 4$ Hz, H-2), 7.25 (d , $J = 4$ Hz, H-4),

7.56 (s , H-5), 7.13 (s , H-7), 2.46 (s , CH_3 -6); ^{13}C NMR (125 MHz, $\text{C}_3\text{D}_6\text{O}$) δ ppm: 166.3 (C-1),

108.8 (C-2), 166.2 (C-3), 109.6 (C-4), 121.4 (C-5), 149.5 (C-6), 124.9 (C-7), 163.2 (C-8), 191.7

(C-9), 182.1 (C-10), 136.4 (C-11), 110.0 (C-12), 114.0 (C-13), 134.2 (C-14), 21.9 (CH₃-6) (Liu et al. 2013).

EI Mass spectrum

IR spectrum

DSF-1.IRS: DSF-1/Tensangmu/MIC
Date: 04/08/2014 Time: 10:33:33 NScans: 10
Type: HYPER IR User: ZUBAIR AHMED Detector: standard
Abscissa: 1/cm Ordinate: %T Apodization: Happ
Min: 400.20 Max: 3999.12 Range: 1/cm
Ndp: 3733 Data Interval: 0.96434 Resolution: 2.0

^1H NMR (300 MHz, $\text{C}_3\text{D}_6\text{O}$)

¹³C NMR (125 MHz, C₃D₆O)

trans-Methyl-*p*-coumarate (**5**) [CAS No 19367-38-5]

trans-Methyl-*p*-coumarate (**5**)

White crystalline (4 mg); melting point: 138 °C; EI MS m/z : 178 [M]⁺; molecular formula:

C₁₀H₁₀O₃; ¹H NMR (300 MHz, C₃D₆O) δ ppm: 6.34 (*d*, J = 15.9 Hz, H-2), 7.59 (*d*, J = 15.9 Hz, H-3), 7.54 (*d*, J = 8.7 Hz, H-5 and H-9), 6.88 (*d*, J = 8.7 Hz, H-6 and H-8), 3.70 (*s*, OCH₃-1); ¹³C NMR (125 MHz, C₃D₆O) δ ppm: 167.8 (C-1), 115.3 (C-2), 145.3 (C-3), 126.9 (C-4), 130.9 (C-5 and C-9), 116.6 (C-6 and C-8), 159.0 (C-7), 51.4 (OCH₃-1) (Chiang et al. 2003).

EI Mass spectrum

ICCBS-HEJ/LAB-104/
3/4/2014

Page 1

File: DSF-2
Sample: TENSANGMU L. TAMANG / DR. M. IQBAL
Instrument: JEOL JMS 600-H
Inlet: My Inlet
Date Run: 03-04-2014 (Time Run: 14:42:55)
Ionization mode: EI+

IR spectrum

DSF-2.IRS: DSF-2/Tensangmu/MIC
Date: 04/08/2014 Time: 10:43:40 NScans: 10
Type: HYPER IR User: ZUBAIR AHMED Detector: standard
Abscissa: 1/cm Ordinate: %T Apodization: Happ
Min: 400.20 Max: 3999.12 Range: 1/cm
Ndp: 3733 Data Interval: 0.96434 Resolution: 2.0

^1H NMR (300 MHz, $\text{C}_3\text{D}_6\text{O}$)

Tengansmu / Dr. Iqbal / DSF-2
1H

AVANCE AV - III
300 MHz, LAB # 116

```

NAME          jan15-14
EXPNO         2
PROCNO        1
Date_         20140115
Time_         14.11
INSTRUM       Spect
PROBHD        5 mm BBO BB-1H
PULPROG       zg30
TD            32768
SOLVENT       Acetone
NS            32
DS            0
SWH           6009.615 Hz
FIDRES        0.183399 Hz
AQ            2.7263477 sec
RG            203
DW            83.200 usec
DE            6.50 usec
TE            300.0 K
D1            1.50000000 sec
TD0           1

===== CHANNEL f1 =====
NUC1          1H
P1            12.50 usec
PL1           0.00 dB
PL1W          13.16228485 W
SFO1          300.1324010 MHz
SI            16384
SF            300.1300067 MHz
WDW           EM
SSB           0
LB            0.30 Hz
GB            0
PC            4.00
    
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¹³C NMR (125 MHz, C₃D₆O)

p-Hydroxybenzaldehyde (6) [CAS No 123-08-0]

p-Hydroxybenzaldehyde (6)

White crystalline (2 mg); melting point: 116 °C; EI MS m/z : 121[M-H]⁺; molecular formula:

C₇H₆O₂; ¹H NMR (300 MHz, C₃D₆O) δ ppm: 7.79 (*d*, *J* = 8.7 Hz, H-2 and H-6), 7.00 (*d*, *J* = 8.7, H-3 and H-5), 9.84 (*s*, HCO); ¹³C NMR (75 MHz, C₃D₆O) δ ppm: 129.5 (C-1), 132.6 (C-2 and C-6), 116.5 (C-3 and C-5), 163.7 (C-4), 190.8.

EI Mass spectrum

IR spectrum

^1H NMR (300 MHz, $\text{C}_3\text{D}_6\text{O}$)

Targansmu / Dr. Iqbal / DSF-3
 ^1H

AVANCE AV - III
300 MHz, LAB # 116

NAME jan15-14
EXPNO 4
PROCNO 1
Date_ 20140115
Time 14.46
INSTRUM Spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT Acetone
NS 32
DS 0
SWH 6009.615 Hz
FIDRES 0.183399 Hz
AQ 2.7263477 sec
RG 203
DW 83.200 usec
DE 6.50 usec
TE 300.0 K
D1 1.50000000 sec
TD0 1

===== CHANNEL f1 =====
NUC1 ^1H
P1 12.50 usec
PL1 0.00 dB
PL1W 13.16228485 W
SFO1 300.1324010 MHz
SI 16384
SF 300.1300067 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 4.00

^{13}C NMR (75 MHz, $\text{C}_3\text{D}_6\text{O}$)

Ursolic acid (7) [CAS No 77-52-1]

Ursolic acid (7)

White crystalline (2 mg); melting point: 288 °C; EI MS m/z : 456 $[\text{M}]^+$; molecular formula:

$\text{C}_{30}\text{H}_{48}\text{O}_3$; ^{13}C NMR (125 MHz, $\text{C}_3\text{D}_6\text{O}$) δ ppm: 39.4 (C-1), 27.9 (C-2), 78.6 (C-3), 39.8 (C-4), 56.1 (C-5), 19.0 (C-6), 33.5 (C-7), 39.8 (C-8), 48.4 (C-9), 37.7 (C-10), 23.8 (C-11), 122.9 (C-12), 144.9 (C-13), 42.4 (C-14), 28.3 (C-15), 24.0 (C-16), 49.5 (C-17), 53.5 (C-18), 42.1 (C-19),

42.1 (C-20), 33.3 (C-21), 39.2 (C-22), 28.6 (C-23), 15.7 (C-24), 16.2 (C-25), 17.5 (C-26), 23.6 (C-27), 176.5 (C-28), 15.8 (C-29), 21.4 (C-30) (Babalola & Shode 2013).

EI Mass spectrum

IR spectrum

^1H NMR (300 MHz, $\text{C}_3\text{D}_6\text{O}$)

Tengansmu / Dr. Iqbal / HP2I
1H

AVANCE AV - III
300 MHz, LAB # 116

^{13}C NMR (125 MHz, $\text{C}_3\text{D}_6\text{O}$)

5-(Hydroxymethyl)-furan-2-carbaldehyde (**8**) [CAS No 67-47-0]

5-(Hydroxymethyl)-furan-2-carbaldehyde (**8**)

Yellowish brown (9 mg); melting point: 35 °C; EI MS m/z : 126 $[\text{M}]^+$; molecular formula:

$\text{C}_6\text{H}_6\text{O}_3$; ^1H NMR (500 MHz, $\text{C}_3\text{D}_6\text{O}$) δ ppm: 7.35 (d , $J = 3.5$ Hz, H-3), 6.56 (d , $J = 3.5$ Hz, H-4), 9.58 (s , HCO-2), 4.63 (H-5'); ^{13}C NMR (75 MHz, $\text{C}_3\text{D}_6\text{O}$) δ ppm: 153.3 (C-2), 123.7 (C-3), 110.1 (C-4), 162.8 (C-5), 178.0 (HCO-2), 57.5 (C-5') (Etse et al. 1988).

EI Mass spectrum

ICCBS-HEJ[LAB-104]
11/26/2013

Page 1

File: D2AETS
Sample: TAMANG/DR.IQBAL
Instrument: JEOL JMS 600-H
Inlet: My Inlet

Date Run: 11-26-2013 (Time Run: 14:56:24)

Ionization mode: EI+

IR spectrum

¹H NMR (500 MHz, C₃D₆O)

TENSANGMU L.TAMANG/DR.IQBAL/D2AET5/C3D6O
ICCBS/U.O.K

AVANCE AV-500
LAB NO: 109-B

^{13}C NMR (75 MHz, $\text{C}_3\text{D}_6\text{O}$)

β -Sitosterol glucoside (9) [CAS No 474-58-8]

β -Sitosterol glucoside (9)

White amorphous (12 mg); melting point: 286 °C; EI MS m/z : 414 $[\text{M-Glu}]^+$; molecular formula: $\text{C}_{35}\text{H}_{59}\text{O}_6$; ^{13}C NMR (150 MHz, CD_3OD) δ ppm: 39.7 (C-1), 29.3 (C-2), 79.9 (C-3), 41.1 (C-4), 141.8 (C-5), 122.8 (C-6), 33.0 (C-7), 33.2 (C-8), 51.7 (C-9), 38.5 (C-10), 22.2 (C-11), 41.1 (C-12), 43.5 (C-13), 58.1 (C-14), 25.3 (C-15), 27.1 (C-16), 57.4 (C-17), 12.4 (C-18), 19.4 (C-19), 37.4 (C-20), 19.5 (C-21), 35.1 (C-22), 30.7 (C-23), 47.2 (C-24), 30.3 (C-25), 20.3 (C-26), 19.9

(C-27), 24.1 (C-28), 12.4 (C-29), 102.4 (C-1'), 71.6 (C-2'), 77.8 (C-3'), 75.1 (C-4'), 78.0 (C-5'), 62.8 (C-6') (Yayli et al. 2003).

EI Mass spectrum

IR spectrum

^1H NMR (500 MHz, CD_3OD)

^{13}C NMR (150 MHz, CD_3OD)

In vitro antileishmanial activity

Leishmania major promastigotes were grown in bulk in modified Novy-MacNeal-Nicolle biphasic medium using normal physiological saline. *Leishmania* promastigotes were cultured with Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, St. Louis, USA), supplemented with 10% heat inactivated Fetal Calf Serum (FCS) (PAA Laboratories GmbH, Austria). Parasites at log phase were centrifuged at 2000 rpm for 10 min and washed three times with saline. Parasites were diluted with fresh culture medium to a final density of 1×10^6 cells/mL.

In a 96-well micro titer plate, 180 μL medium was added in different wells. The extract and compounds were dissolved in phosphate buffer saline (pH 7.4 containing 0.5% MeOH and

0.5% DMSO) to make a stock concentration of 1000 mg/mL. The stock solution (20 μ L) was added in medium and serially diluted to obtain working concentrations (1-100 μ g/mL). Parasite culture (100 μ L) was added in all wells. Two rows were left for negative and positive controls. Negative controls received only medium while the positive controls contained different concentrations of standard antileishmanial compounds namely amphotericin B (MP Biomedical Inc.) and pentamidine (ICN Biomedical Inc). The plate was incubated at 25 °C for 72 h. The culture was examined microscopically on an improved Neubaure counting chamber and IC₅₀ values were calculated using Software Ezfit 5.03 Perella Scientific. All assays were run in duplicate (Choudhary et al. 2005). Table S1 shows the data resulted from the antileishmanial activity.

Table S1. *In vitro* antileishmanial activity of the different extracts and pure compounds isolated from *A. spicatum*.

Treatments	IC ₅₀ (μ g/mL) $\pm$ S.D.	SI
<i>n</i> -Hexane extract	> 100	-
Dichloromethane extract (pH 2.5)	27.10 $\pm$ 0.0	36.9 ^a
Dichloromethane extract (pH 7)	> 100	-
Ethyl acetate extract	> 100	-
<i>n</i> -Butanol extract	> 100	-
Indaconitine (1)	> 100	-
Chasmaconitine (2)	56.3 $\pm$ 2.1	0.53 ^b
Ludaconitine (3)	36.1 $\pm$ 3.4	0.83 ^b
Amphotericin B (Standard)	0.29 $\pm$ 0.05	-
Pentamidine (Standard)	5.09 $\pm$ 0.09	-

$SI^a = IC_{50}$ value for extract on brine shrimp divide by the IC_{50} value for the extract on *Leishmania major*

$SI^b = IC_{50}$ value for compound on 3T3 cells divide by the IC_{50} value for the extract on *Leishmania major*

Brine shrimp cytotoxicity

Test plant extracts were dissolved in pure dimethyl sulfoxide (DMSO) and re-dissolved in sea water to make concentrations were 100, 500, 1000 $\mu\text{g/mL}$. After hatching and maturation of *Artemia salina*, 10 larvae were placed in each plate using pipette and incubated at 25-27°C for 24 h under illumination. After 24 h, the numbers of dead naupli in each plate were counted. The experiment was done in triplicate. DMSO was used as negative control and thymol as positive control. The percentage lethality was determined by comparison of mean surviving larvae of the test and control Plates (Meyer et al. 1982).

MCF7, 3T3, HeLa and PC3 cell lines cytotoxicity

Cytotoxicity of compounds was evaluated by using the MTT assay (Mosmann 1983). The cell-lines were cultured in Dulbecco's Modified Eagle's Medium, supplemented with 5% of fetal bovine serum (FBS), 100 IU/mL of penicillin, and 100 $\mu\text{g/mL}$ of streptomycin. Then, 1×10^4 cells/well loaded into each well of 96-well plates. After overnight incubation, it was added with different concentrations of compounds. After 72 hrs, MTT was added to each well and incubated further for 4 hrs, and then the absorbance was taken at 570 nm.

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