Supporting Information

Profiling of Alkaloids and Eremophilanes in Miracle Tea (*Packera candidissima* and *P. bellidifolia*) Products

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Figure S1. Photographs of *Packera candidissima*, *P. bellidifolia*, and their commercial presentations A) Plants of *Packera candidissima* in forest opening west of Creel, Chihuahua (Bye 34760, sample PC-2); B) Crude drug; C) Dried plants of “chucaca” or “lechugilla” for sale in Mercado La Reforma, Chihuahua City. D) Miracle tea (“té milagro”) products. Commercial presentations include dried whole plants, dried leaves, alcoholic extracts and cream preparations. E) Herbal drug: Selected dried leaves and stems without roots of “calancapatle”, *P. bellidifolia* (syn., *Senecio vulneraria*; Bye 34906, sample PCC-1).

Figure S2. UV spectral for compounds **1**, **2** and **8**.

Figure S3. ^1H NMR (300 MHz, CDCl_3) spectrum of neoadenostylone (**3**).

Figure S4. ^{13}C NMR (75 MHz, CDCl_3) spectrum of neoadenostylone (**3**).

Figure S5. ¹H NMR (300 MHz, CDCl₃) spectrum of 6-(2- α-methylbutanoyloxy)-9-oxo-1(10)-furanoeremophilene (**4**).

Figure S6. ^{13}C NMR (300 MHz, CDCl_3) spectrum of 6-(2- α -methylbutanoyloxy)-9-oxo-1(10)-furanoeremophilene (**4**).

Figure S7. ^1H NMR (300 MHz, CDCl_3) spectrum of epineoadenostylone (**5**).

Figure S8. ^{13}C NMR (75 MHz, CDCl_3) spectrum of epineoadenostylone (5).

Figure S9. ^1H NMR (300 MHz, CDCl_3) spectrum of epicacalone (**6**).

Figure S10. ^{13}C NMR (75 MHz, CDCl_3) spectrum of epicacalone (**6**).

Figure S11. ¹H NMR (500 MHz, CDCl₃) spectrum of 2-hydroxyneoadenostylone (7).

Figure S12. ^{13}C NMR (500 MHz, CDCl_3) spectrum of 2-hydroxyneoadenostylone (7).

Figure S13. GC chromatograms of alkaloid extracts.

Figure S14. GC chromatogram of hexane-soluble extracts.

(A)

(B)

Figure S15. GC chromatogram of samples PC-2 and PBC-3.

Figure S16. Composition of compounds 1, 2, and 8 profiled qualitatively by HPLC in alkaloid extracts.

Figure S17. Composition of compounds **3** and **5** profiled qualitatively by HPLC in hexane-soluble extracts.

Figure S18. HPLC-PDA chromatograms of pyrrolizidine alkaloid extracts (214 nm).

Figure S19. HPLC-PDA chromatograms of hexane-soluble extracts (280 nm).

Table S1. Sample identification chart.

Plant ^a	Voucher	Procedence ^b , Date collected or purchased, Part used	Origin ^c	Sample/Code
Pc	Bye 34760	CHH: Bocoyna, Creel/ 2006 08 31/ Whole plant	F	01/PC-1
Pc	Bye 35038	CHH: Bocoyna, Estación Sánchez/ 2007 05 23/ Whole plant	F	02/PC-2
Pc	Linares 1797	CHH: Chihuahua City/ 2006 10 22/ Whole plant	M	03/PCC-2
Pc	Bye 35041	CHH: Bocoyna, San Juanito; Noritari/ 2007 05 24/ Whole plant	F	04/PC-3
Pc	Bye 17179	CHH: Chihuahua City, Market La Reforma/ 1989 08 23/ Aerial Parts	M	05/PCC-1
Pc	Bye 35049	CHH: Chihuahua City, Market La Reforma/ 2007 05 26/ Aerial parts	M	06/PCC-4
Pc	Bye 35056	CHH: Chihuahua City, Market La Reforma/ 2007 05 26/ Aerial parts	M	07/PCC-5
Pc	Bye 35059	CHH: Chihuahua City, Market La Reforma/ 2007 05 26/ Aerial parts	M	08/PCC-6
Pc	Bye 35063	CHH: Chihuahua City, Arámbula/ 2007 05 26/ Aerial parts	M	09/PCC-7
Pc	Linares 1811	CHH: Chihuahua City; Market La Reforma/ 2006 10 22/Alcoholic extract	M	10/PPS-4
Pc	Linares 1810	CHH: Chihuahua City; Arámbula/ 2006 10 22/ Aerial parts	M	11/PPS-2
Pc	Bye 35062	CHH: Chihuahua City; Arámbula/ 2007 05 26/ Tea bag	M	12/PPS-6
Pc	Bye 35065	CHH: Chihuahua City; Arámbula/ 2007 05 26/ Cream	M	13/PPS-7
Pb	Bye 34679	DF: Mexico City; La Magnolia/ 2005 10 06/ Aerial parts	M	14/PBC-1
Pb	Bye 34680	DF: Mexico City; Herbal shop La Magnolia/ 2005 10 06/ Aerial parts	M	15/PBC-2
Pb	Bye 34680b	DF: Mexico City; Herbal shop La Magnolia/ 2005 10 06/ Aerial parts	M	16/PBC-3
Pb	Bye 34680c	DF: Mexico City; Herbal shop La Magnolia/ 2005 10 06/ Aerial parts	M	17/PBC-4
Pb	Bye 35895	MXC: Municipio Amecameca, Paso de Cortés/ 2008 03 09/ Whole plant	F	18/PB-1
Pc	Bye 34906	NL: Monterrey/ 2006 10 27/ Aerial parts	M	19/PCC-3
	L0609422	QRT: Santiago de Querétaro, Herbal shop/ 2006 08 20/ Alcoholic extract	M	20/PPS-1
	L0505242	QRT: Santiago de Querétaro; Herbal shop/ 2006 08 20/ Alcoholic extract	M	21/PPS-3
	L0505288	QRT: Santiago de Querétaro; Herbal shop/ 2006 08 20/ Leaves	M	22/PPS-5

^a Pc: *Packera candidisima*, Pb: *Packera bellidifolia*; ^bCHH: Chihuahua, DF: Distrito Federal, MXC: Estado de México, NL: Nuevo León, QRT: Querétaro. ^cF: Field, M: Market.

Table S2. Yields of free and N-oxides PAs. ^a

Sample	% Free PAs	% N oxides
Whole plant		
PC-1	0.813	0.555
PC-2	0.033	0.024
PC-3	0.021	0.016
PB-1	0.010	0.008
Aerial parts		
PCC-1	0.942	0.480
PCC-2	0.178	0.276
PCC-3	0.174	0.291
PCC-4	0.045	0.036
PCC-5	0.023	0.019
PCC-6	0.002	0.001
PCC-7	0.145	0.139
PBC-1	0.216	0.264
PBC-2	0.119	0.206
PBC-3	0.366	0.205
PBC-4	0.565	0.306
Phytopharmaceutical samples		
PPS-1	0.001	0.010
PPS-2	0.001	0.006
PPS-3	0.042	0.041
PPS-4	0.046	0.055
PPS-5	0.001	0.002
PPS-6	0.001	0.005
PPS-7	0.0005	0.0004

^aYield, w/w in terms of dry starting material.

Table S3. Hypothetical alkaloid intake from consumption of miracle tea.

Person	mg/kg	mg	Dose/Weight of plant material (g)				
			<i>P. candidissima</i> ^a		<i>P. bellidifolia</i> ^a		Alcoholic extract ^a
			PC-1 ^b	PCC-1 ^c	PB-1 ^b	PBC-4 ^c	PPS-1
10 kg child	10	100	24.3	17.8	31.9	55.9	8571.4
60 kg adult	10	600	146.0	106.5	191.3	335.2	33571.4

^aFor sample codes, see Table S1 (Supporting information), ^bWhole plant, ^cAerial parts.

Table S5. HPLC-PDA calibration curves^a and limits of detection (LOD) for standard samples.

Compound	Slope	Intercept	Correlation coefficient	Linearity (µg/mL)	LOD (µg/mL)
1	5.08×10^6	6.43×10^5	0.9947	$(5.08 \pm 0.22) \times 10^6$	0.5
2	5.01×10^6	4.72×10^5	0.9927	$(5.01 \pm 0.25) \times 10^6$	0.5
8	2.46×10^7	1.05×10^6	0.9928	$(2.47 \pm 0.13) \times 10^7$	0.5

^aFor each curve the equation is $y = ax + b$, where y is the peak area, x is the concentration of the analyte (µg/mL), a is the slope, b is the intercept.

General Experimental Procedures. Optical rotations were measured in CHCl_3 on a Perkin-Elmer 241 polarimeter. IR spectra were obtained in KBr on a Perkin-Elmer 1600 FT spectrophotometer. 1D and 2D NMR spectra were measured from CDCl_3 solutions containing TMS as the internal standard at 300 MHz for ^1H and 75.4 MHz for ^{13}C on a Varian *Unity-Inova* spectrometer. FABS were recorded on a JEOL DX300 mass spectrometer in the positive mode using NBA as the matrix. Open Column chromatography was carried out on Merck silica gel 60 (70-230 mesh ASTM) and TLC on Merck silica gel 60 F254 plates. HPLC analysis was performed on a Waters equipment (Millipore Co., Milford, MA, U.S.) consisting of a solvent distribution system Model 600 E and a multiple UV wavelength photo-diode array detector (Model 996), linked to a computerized control system (Optiflex 466/LE, DELL). The uploading and processing of the chromatographic information was done using the Empower 2 software (Waters). In preparative HPLC, the technique of heart cutting and independently reinjected in the apparatus operating in the recycle mode to achieve total homogeneity after 20 consecutive cycles employing the same isocratic elution indicated for each compound.

Neoadenostylone (3): ^1H NMR δ 7.38 (d, $J = 1.0$ Hz, H-12), 7.00 (t, $J = 4.1$ Hz, H-1), 6.42 (s, H-6), 6.27 (qq, $J = 7.3, 1.4$ Hz, H-3'), 2.24 (m, H-2), 2.11 (dq, $J = 7.3, 1.4$ Hz, H-4'), 1.98-2.00 (m, H-4), 1.99 (dq, $J = 3.0, 1.4$ Hz, H-5'), 1.87 (d, $J = 1.1$ Hz, H-13), 1.52 (m, H-3a), 1.44 (m, H-3b), 1.16 (s, H-14), 0.97 (d, $J = 6.9$ Hz, H-15); ^{13}C NMR δ 176.3 (C-9), 166.7 (C-1'), 146.5 (C-8), 145.7 (C-12), 141.4 (C-3'), 141.1 (C-10), 138.0 (C-1), 135.8 (C-7), 126.8 (C-2'), 121.0 (C-11), 73.9 (C-6), 46.7 (C-5), 38.0 (C-4), 28.0 (C-3), 25.3 (C-2), 20.7 (C-5'), 17.7 (C-15), 16.2 (C-4'), 16.0 (C-14), 8.6 (C-13).

6-(2- α -methylbutanoyloxy)-9-oxo-1(10)-furanoremodophylene (4): ^1H NMR δ 7.40 (d, $J = 1.2$ Hz, H-12), 6.99 (t, $J = 4.4$ Hz, H-1), 6.35 (s, H-6), 2.35 (m, H-2'), 2.29 (m, H-2), 2.24 (m, H-3'), 1.93 (d, $J = 0.9$ Hz, H-13), 1.80 (m, H-4), 1.56-1.49 (m, H-3), 1.11 (s, H-14), 1.02 (m, brs, H-5'), 1.00 (brs, H-4'), 0.99 (d, $J = 6.6$ Hz, H-15); ^{13}C NMR δ 177.0 (C-9), 172.7 (C-1'), 146.8 (C-8), 145.9 (C-12), 141.3 (C-10), 138.2 (C-1), 136.0 (C-7), 121.0 (C-11), 74.2 (C-6), 46.6 (C-5), 43.4 (C-2'), 37.1 (C-4), 27.8 (C-3), 25.5 (C-2), 24.8 (C-3'), 22.6 (C-5'), 22.3 (C-4'), 17.5 (C-15), 15.6 (C-14), 8.7 (C-13).

Epineoadenostylone (5): ^1H NMR δ 7.41 (d, $J = 1.2$ Hz, H-12), 6.99 (t, $J = 4.1$ Hz, H-1), 6.35 (s, H-6), 6.30 (qq, $J = 7.5, 1.5$ Hz, H-3'), 2.18 (s, H-2), 2.11 (dq, $J = 3.0, 1.5$ Hz, H-4'), 1.99 (t, $J = 1.7$ Hz, H-4), 1.93 (dq, $J = 5.6, 4.8$ Hz, H-5'), 1.88 (d, $J = 0.9$ Hz, H-13), 1.38-1.58 (m, H-3), 1.11 (s, H-14), 1.04 (d, $J = 6.6$ Hz, H-15); ^{13}C NMR δ 176.7 (C-9), 167.0 (C-1'), 145.9 (C-3'), 141.6 (C-1), 141.3 (C-8 and C-10), 137.9 (C-12), 135.8 (C-7), 126.9 (C-2'), 121.2 (C-11), 73.9 (C-6), 46.6 (C-5), 37.9 (C-4), 27.9 (C-2), 25.1 (C-4'), 22.6 (C-5'), 17.5 (C-14), 15.8 (C-15), 8.40 (C-13).

Epicalone (6): ^1H NMR δ 7.33 (q, $J = 0.9$ Hz, H-12), 2.54 (ddd, $J = 18.9, 6.9, 2.6$ Hz, H-2b), 2.31 (ddd, $J = 18.9, 9.3, 0.5$ Hz, H-2a), 2.23 (d, $J = 0.9$ Hz, H-13), 1.74-1.78 (m, H-9), 1.66-1.69 (m, H-3b), 1.64 (s, H-14), 1.88 (d, $J = 0.9$ Hz, H-13), 1.38-1.58 (m, H-3a), 1.11 (s, H-14), 1.04 (d, $J = 6.6$ Hz, H-15); ^{13}C NMR δ 175.0 (C-1), 161.5 (C-6), 145.2 (C-8), 144.3 (C-12), 140.4 (C-7), 130.7 (C-5), 120.2 (C-11), 72.3 (C-10), 30.3 (C-3), 28.5 (C-4), 27.2 (C-14), 21.6 (C-2), 21.4 (C-15), 16.0 (C-9), 8.8 (C-13).