

INTRASPECIFIC VARIABILITY OF *Holostylis reniformis*: CONCENTRATION OF LIGNANS, AS DETERMINED BY MACERATION AND SUPERCRITICAL FLUID EXTRACTION (SFE-CO₂), AS A FUNCTION OF PLANT PROVENANCE AND PLANT PARTS

Gislaine F. Martins^a, Marcos D. P. Pereira^a, Lucia M. X. Lopes^{*a}, Tito da Silva^b, Paulo de T. Vieira e Rosa^c, Fernanda P. Barbosa^c, Gisele B. Messiano^d and Antoniana U. Krettli^e

^aInstituto de Química, Universidade Estadual Paulista, 14801-970 Araraquara – SP, Brasil

^bCentro de Ciências Sociais, Saúde e Tecnologia, Universidade Federal do Maranhão, 65900-410 Imperatriz – MA, Brasil

^cInstituto de Química, Universidade Estadual de Campinas, 13083-970 Campinas – SP, Brasil

^dInstituto Federal de São Paulo, 15990-040 Matão – SP, Brasil

^eInstituto René Rachou, FIOCRUZ, 30190-002 Belo Horizonte – MG, Brasil

Table 1S. Composition of extracts determined by HPLC-DAD and HPLC-DAD-ESI/MS^a

Variable ^b	Lignan	[M+H] ⁺ m/z	t _R (min)	Extracts (%)													
				E-1	E-2	E-4	E-5	E-7	E-8	E-10	E-11	E-13	E-14	E-16	E-17	E-20	E-23
4			6.75	0.00	1.59	4.42	0.00	5.05	0.00	0.00	0.00	5.67	3.03	9.91	9.14	0.00	6.10
6			7.47	0.00	0.00	1.82	0.00	0.00	0.00	2.29	0.00	0.00	0.00	7.28	0.00	0.00	0.00
7			7.78	0.00	0.00	0.00	6.82	0.00	0.00	0.00	0.00	0.00	1.07	0.00	0.00	0.00	0.00
8	13	341	7.94	2.87	0.00	6.24	0.00	3.36	0.00	6.69	5.73	4.67	0.00	0.00	0.00	0.00	0.00
10			8.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.58	0.00	7.36	8.40	7.10
11	10	357	8.54	2.73	5.05	3.59	10.30	2.95	7.01	6.00	4.02	1.77	0.00	6.95	0.00	0.00	0.00
12			8.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.77	55.60	0.00	13.79	53.57	14.24
13			8.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.39	0.00	0.00	0.00
14	9	357	9.09	3.91	3.45	7.07	8.32	5.74	3.64	6.89	7.66	0.00	0.00	7.67	0.00	0.00	0.00
18			11.03	0.00	0.00	0.00	5.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	1	371	11.06	37.12	39.37	13.10	6.25	33.34	39.18	8.39	12.22	32.04	9.31	7.23	0.00	14.71	0.00
21	4	371	12.72	12.66	14.84	10.56	13.27	11.67	13.54	11.56	9.76	11.28	3.06	8.39	0.00	4.94	0.00
26			15.26	0.00	0.00	4.60	0.00	0.00	5.26	2.98	2.89	0.00	8.09	8.94	1.47	0.00	0.00
27			15.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.75	0.00	0.00	0.00	0.00	0.00
28	7	371	15.98	13.27	0.00	4.70	0.00	11.89	11.73	4.44	2.59	5.60	1.42	3.29	0.00	2.22	0.00
29	3	355	16.66	0.00	14.28	3.53	6.27	0.00	1.70	2.89	2.67	1.59	8.36	1.89	58.72	11.66	71.08
30			18.73	3.21	0.00	0.00	0.00	0.00	0.00	0.00	18.79	2.25	0.00	0.00	0.00	0.00	0.00
32	5	355	18.82	0.00	1.14	4.79	0.00	1.99	0.00	2.82	0.00	0.00	0.00	1.54	0.00	0.00	0.00
33	2	355	20.88	13.46	14.39	20.57	38.22	11.28	13.63	18.79	25.84	12.47	3.44	15.69	3.10	2.41	0.00
36	8	339	23.49	0.00	0.00	1.83	0.00	0.00	0.00	2.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37	12	357	27.95	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
38	6	339	31.23	0.00	1.03	1.94	0.00	0.00	0.00	1.89	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39	16	357	31.24	0.00	0.00	1.42	0.00	0.00	0.00	1.86	1.62	0.00	0.00	0.00	0.00	0.00	0.00
40	15	373	33.25	0.00	0.00	1.38	0.00	0.00	0.00	1.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
41	14	339	33.29	0.00	1.16	0.00	0.00	0.00	0.00	1.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00

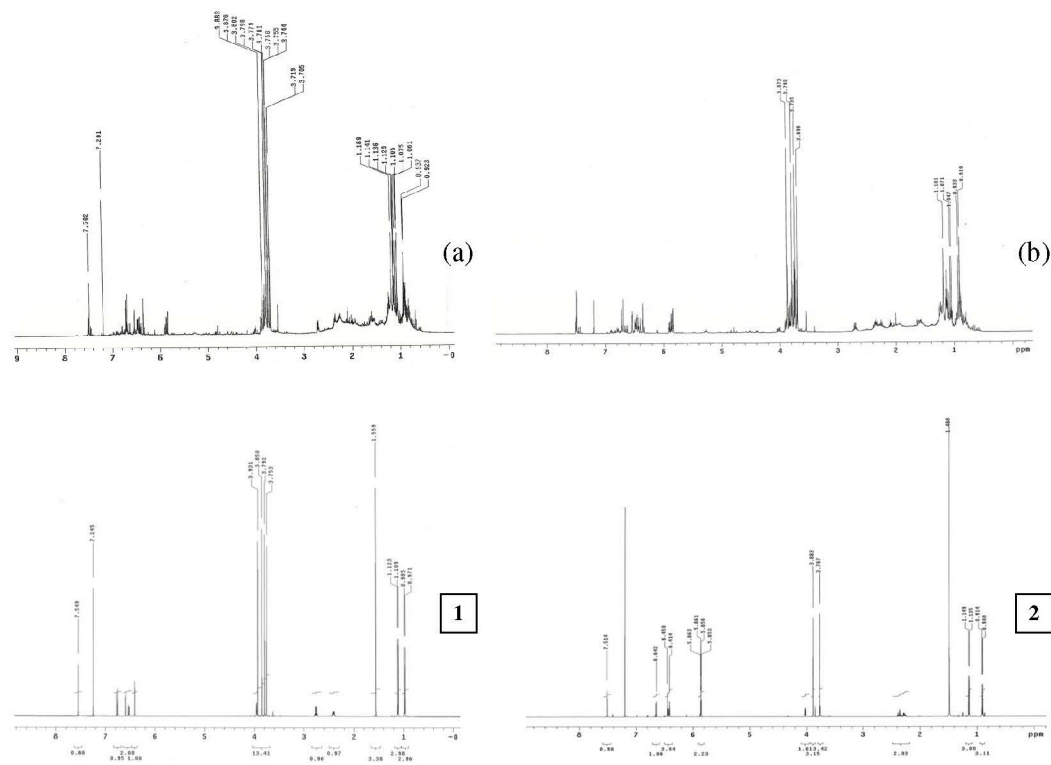
^a t_R: Retention times; composition expressed as a percentage in the range of 6.18 to 33.29 min; for extract codes, see Table 1. ^b Selected variables: lignans and compounds whose concentrations were at least 5.0% in one of the analysed extracts.

Table 2S. Scores for three principal components (PCs) for the dataset of hexane solutions of extracts from stems and roots of *H. reniformis* by GC-MS

Samples ^a	Factor 1	Factor 2	Factor 3
E-1	-0.613641	-23.172598	2.023694
E-2	-4.602954	20.126415	14.383844
E-4	39.004322	-11.543629	6.696213
E-5	22.525829	10.712484	-16.207546
E-7	-16.784327	-21.461687	0.604645
E-8	-14.503395	12.219756	9.707336
E-10	-6.719095	-1.204995	-2.461890
E-11	8.812049	25.054316	-38.985374
E-13	-50.409611	-29.680290	-9.987580
E-14	-24.718164	35.679787	21.992687
E-16	58.008984	-16.729565	12.233972

^a For extract codes, see Table 1.**Table 3S.** Scores for three principal components (PCs) for the dataset of hexane solutions of extracts from stems and roots of *H. reniformis* by HPLC-DAD-ESI/MS

Samples ^a	Factor 1	Factor 2	Factor 3
E-1	-22.968166	-0.334571	14.723046
E-2	-12.124438	7.029879	17.934275
E-4	-12.556252	6.110610	-9.507031
E-5	-13.031762	11.809308	-23.040548
E-7	-20.528162	0.110139	12.207561
E-8	-22.504902	0.219117	15.972814
E-10	-11.484458	6.135619	-13.343925
E-11	-14.695626	6.966284	-14.297139
E-13	-14.316376	-6.299582	10.262061
E-14	23.812603	-41.885036	-6.918268
E-16	-9.725595	5.473227	-13.969701
E-17	48.379227	19.463631	2.646794
E-20	23.453072	-39.314293	-0.090531
E-23	58.290833	24.515673	7.420588

^a For extract codes, see Table 1.**Figure 1S.** Representative ¹H NMR spectra of root extracts of *H. reniformis* (collected in MG): (a) hexane, (b) SFE-CO₂, and of standards 1 and 2 (CDCl₃, 500 MHz)

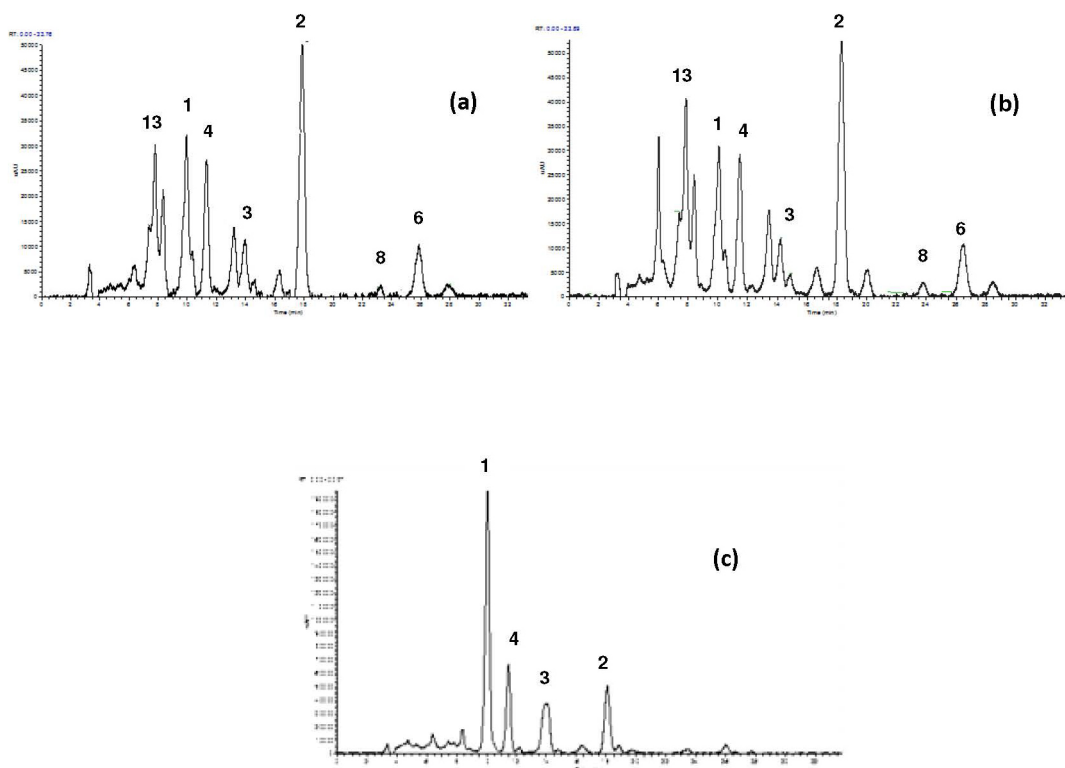


Figure 2S. Representative total ion current (TIC) of hexane (a) and SFE-CO₂ (b) extracts from the roots of *H. reniformis* from plants collected in MA, and SFE-CO₂ (c) from plants collected in MG. Peaks corresponding to the identified lignans are shown (20 V, positive mode, C18, 150 × 3.9 mm, UV detection at 254 nm, and flow rate: 0.8 ml min⁻¹)

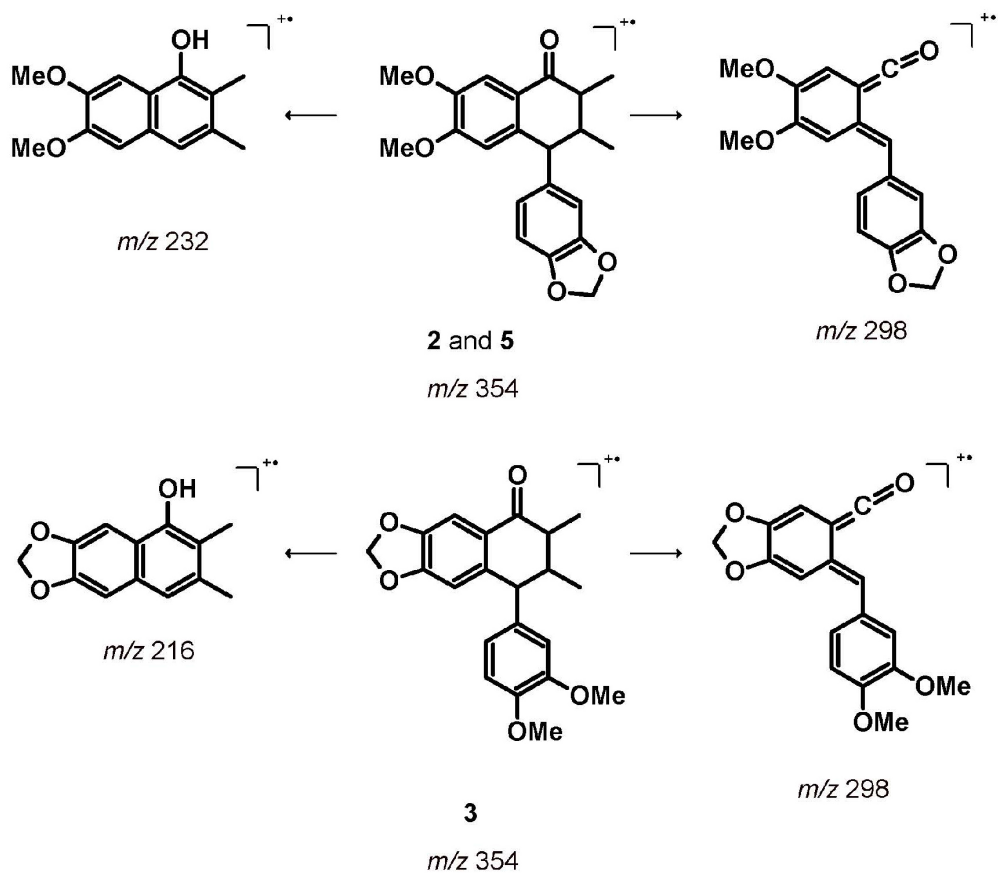


Figure 3S. Key ions obtained by GC-MS and ESI-MS of 2, 3, and 5

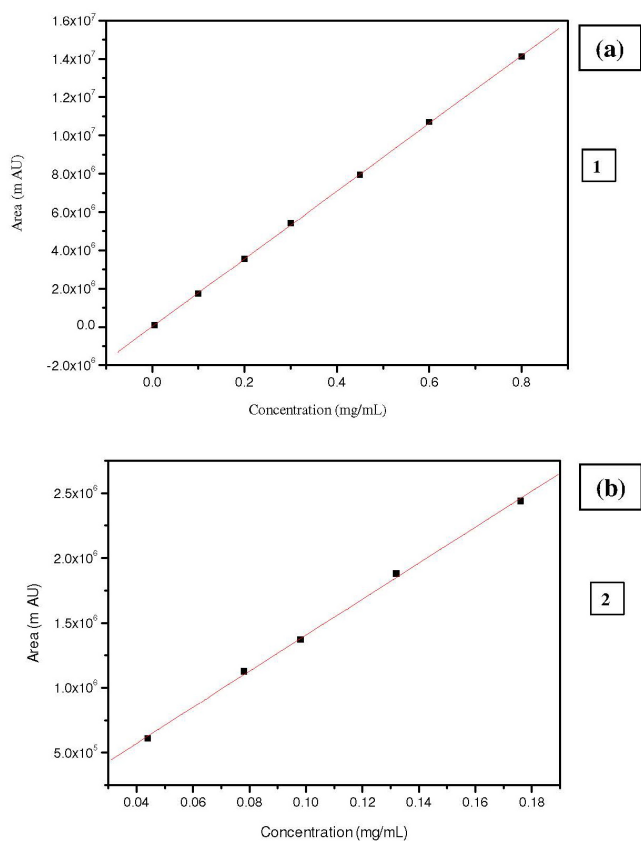


Figure 4S. Linearity of response (m AU) for 1 and 2 standards with HPLC-DAD method. Concentrations of solutions used in the calibration curve: (a) Concentrations of 1: 0.005, 0.1, 0.2; 0.3, 0.45, 0.6, and 0.8 mg/mL; (b) Concentrations of 2: 0.044, 0.078, 0.098, 0.132, and 0.176 mg/mL

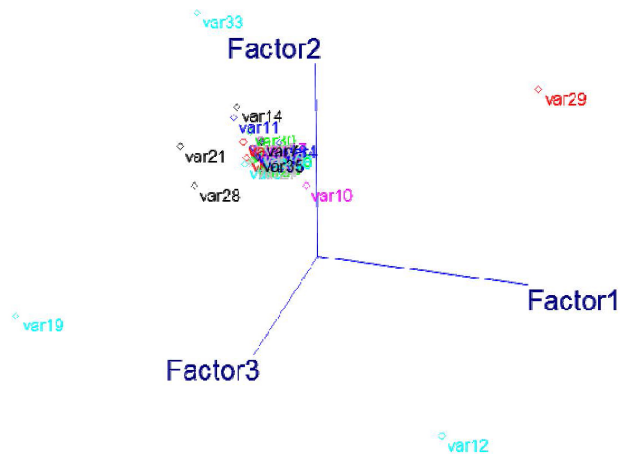


Figure 6S. Loading plot of principal components (PC1, PC2, and PC3) obtained for hexane solutions of extracts from stems and roots of *H. reniformis* by HPLC-DAD-ESI/MS and HPLC-DAD (for variable code, see Table 1S)

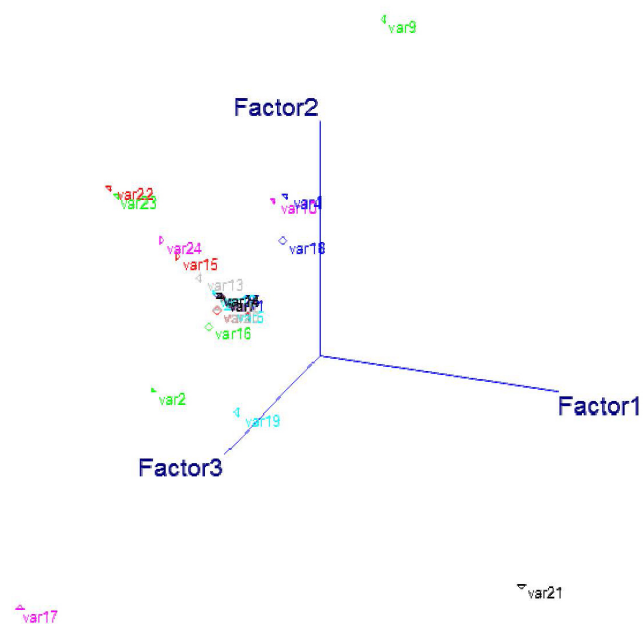


Figure 5S. Loading plot of principal components (PC1, PC2, and PC3) obtained for hexane solutions of extracts from stems and roots of *H. reniformis* by GC-MS (for variable code, see Table 3)