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Article

Chemical Profile and Cytotoxic Activity of Leaf Extracts from *Senna* spp. from Northeast of Brazil

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Methanolic, diethyl ether and *n*-hexane extracts of leaves of four species of *Senna* (*S. gardneri, S. macranthera, S. splendida* and *S. trachypus*) were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Using linear retention indices and mass spectral data, 34 compounds were identified, including fatty acids, flavonoids, terpenoids and steroids that were not reported previously for these species. Additionally, the cytotoxicity of the extracts against different tumor cell lines was determined. The cytotoxicity was then correlated with the chemical composition of the extracts by partial least squares-discriminant analysis (PLS-DA). The *n*-hexane extract of *S. gardneri* and the ethyl ether extract of *S. splendida* were the most active against human colon (59.75 and 31.37%, respectively) and human glioblastoma (52.85 and 48.28%, respectively) cell lines.

Keywords: *Senna* spp., GC-MS, dereplication, cytotoxicity, multivariate analysis

Introduction

The genus *Senna* (Leguminoseae) contains approximately 260 species found in tropical and subtropical regions worldwide and is widely distributed in northeastern, southeastern and southern Brazil.[1-3] *Senna* species are also reported in India, Australia and Africa.[4] The genus is within the tribe Cassieae Bronn and subtribe Cassinae Irwin & Barneby, which also include the genera *Cassia* L. and *Chamaecrista* Moench. The species of *Senna* and *Chamaecrista* were included in the genus *Cassia* before the taxonomic revision of Irwin and Barneby, 1981.[5]

Senna species produce flavonoids, polysaccharides, steroids, chromones, lactones and triterpenes but the most common classes of secondary metabolites are anthraquinones and piperidine alkaloids.[6-8] Based on literature reports, the leaves of *Senna* species have a variety of pharmacological activities. The laxative propriety of anthrone rhein is well known, which is obtained as sennosides primarily from *Cassia acutifolia* and *C. angustifolia* and is metabolized by bacteria from the intestinal tract of humans.[9] *n*-Hexane and methanol extracts of the leaves of *S. macranthera* have strong laxative activity, comparable with that of the positive control bisacodyl, and anti-inflammatory activity, similar to that of diclofenac sodium.[10]

The dimeric indole alkaloid cassiaindoline in the leaves of *S. alata* has significant analgesic and anti-inflammatory activities, and the anthraquinones in the leaves of this species has effective antifungal and bactericidal activities.[11] Lipophilic extracts and anthraquinones obtained from seeds of *C. tora* inhibit EBV-EA activation induced by teleocidin B-4, which may indicate a chemopreventive action.[12] Extracts and the alkaloids cassine and spectaline of *S. spectabilis* have no cytotoxic effect on murine macrophages (J774 cell line),[8] and the extract of *S. sophera* is not cytotoxicity.[13]

Barakol, an anxiolytic agent isolated by acid hydrolysis of *S. siamea* leaves, is toxic to P19 cells and induces apoptosis,[14] but the ethanolic and aqueous extracts of leaves from *C. siamea* (syn. *S. siamea*) were not cytotoxic to KB and Vero cells.[15] Esakkirajan *et al.*[16] obtained spiro[piperidine-4,2'(1'*H*)-quinazolin]-4'(3'*H*)-one from the leaves of *C. auriculata* with an half maximal inhibitory concentration (IC_{50}) value of 25 μg mL^{-1} for human colon cancer cell line HCT15.

The techniques used classically to isolate and identify natural compounds from plants are frequently criticized

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because of the long chromatographic process required, the waste of organic solvents, and often, the isolation of well-known compounds in various species.[17] Therefore, to avoid the re-isolation of known compounds, qualitative analysis on hyphenated systems (dereplication) has a fundamental role in the analysis of metabolic profiles of the most diverse species of plants.[18,19] Additionally, the comparisons of chemical profiles of extracts with different biological activities can be used to indicate hit compounds related to the observed activity. Then, to correlate chemical compounds and biological activity, data can be analyzed by multivariate statistics; typically combining supervised techniques like principal component analysis (PCA) or hierarchical cluster analysis (HCA), and unsupervised techniques, partial least squares (PLS) and related regression techniques.[20,21]

Based on these considerations and the positive and negative results previously reported for the cytotoxicity of extracts from *Senna* and *Cassia* species, 12 extracts from the leaves of four species of *Senna* (*S. gardneri, S. macranthera, S. splendida* and *S. trachypus*) were evaluated for cytotoxic effects on three human cancer cell lines, i.e., OVCAR-8, HCT-116, and SF-295. The chemical profiles of the extracts obtained by gas chromatography coupled to mass spectrometry (GC-MS) were then compared to identify active compounds.

Experimental

Plant material

The leaves of *S. gardneri*, *S. macranthera, S. splendida* and *S. trachypus* were collected in Chapada Ibiapaba, Ceará, Brazil, between August 2010 and March 2012. Plants were identified by Prof Edson de Paula Nunes, Departamento de Biologia of Universidade Federal do Ceará (UFC), and the samples were registered as numbers 47.385, 47.384, 47.387 and 47.377, respectively, and stored in the Herbarium Prisco Bezerra at UFC. After drying at room temperature (ca. 25 °C), 3.0 g of leaves from each species of *Senna* was extracted with 40 mL of organic solvents (*n*-hexane, diethyl ether and methanol, consecutively) for 12 min in an ultrasound bath. The extracts were filtered and then concentrated under vacuum on a rotary evaporator.

Reagents and equipment

The homologous series of C_{12}-C_{40} alkanes, pyridin, MSTFA (*N*-trimethylsilyl-*N*-methyl trifluoroacetamide) and methoxyamine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were analytical grade and were purchased from Merck (Darmstadt, Germany). All solvents used for GC-MS analyses were analytical grade. Methanol, *n*-hexane and diethyl ether were purchased from Tedia (Fairfield, USA). The samples were analyzed on a Shimadzu GC-MS QP2010 (Tokyo, Japan) equipped with an automatic sampler AOC-20Si using an ionization source of 70 eV and fragmentation by electron ionization (EI), GC-MS Solutions software version 1.02 (Tokyo, Japan) and a fused silica capillary column SULPECO DB-5 (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm × 0.25 μm). For statistical analyses, HCA and PLS-DA were performed using Matlab 7.12.0 (MathWorks, Natick, MA, USA) and PLS_Toolbox (Eigenvector Research, Wenatche, USA), respectively.

Extract derivatization

After drying at room temperature (ca. 25 °C), 3.0 g of leaves from each species of *Senna* was extracted with 40 mL of solvents (*n*-hexane, diethyl ether and methanol, consecutively) for 12 min in a sonicator. The extracts were filtered and then concentrated under vacuum. All extracts were treated with the trimethylsilylation reaction. The methanolic extracts were subjected to methoxymation with modifications.[22] Extracts (20 mg each) were added to vials and dissolved in 300 μL of pyridine. Shortly thereafter, 100 μL of methoxyamine hydrochloride (20 mg mL^{-1}) was added to the vials and the derivatization was performed at 30 °C for 90 min. Following derivatization, 150 μL of MSTFA was added and the final solution was placed in a water bath at 37 ºC for 30 min. The *n*-hexane and ethyl ether extracts (20 mg) were dissolved in 300 μL of pyridine. Subsequently, 150 μL of MSTFA was added and the above procedure was repeated. After termination of the reaction, the samples were filtered through membranes (Chromafil® Xtra RC-20/25, with 0.20 μm pores) and stored in 2 mL vials for 24 h at 4 °C before GC-MS analysis.

Gas chromatography coupled to mass spectrometry analyses (GC-MS) of *Senna* spp. extracts and identification of compounds

The samples were analyzed by GC-MS with injector temperature was adjusted to 260 °C. Helium (1 mL min^{-1}) was the carrier gas and injections of 1 μL occurred in split mode (1:10). The oven temperature was kept at 120 °C for 3 min and then programmed to 320 °C at 3 °C min^{-1}. The mass spectrometer operated in EI mode (70 eV) and the acquisition range was *m/z* 40-660. The total time of the analysis was 79.67 min. The trimethylsilane (TMS)

derivatives were identified by comparison of their mass spectra with those in National Institute of Standards and Technology (NIST) or Wiley libraries and Golm Metabolome Database (GMD), requiring at least 90% similarity and experimental linear retention indices (RI) within literature RI values ± 10.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for cell viability

Tumor cell lines OVCAR-8 (ovarian carcinoma), HCT-116 (human colon) and SF-295 (human glioblastoma) were provided by the National Cancer Institute (USA). The extracts from the leaves of *Senna* species were diluted in dimethyl sulfoxide (DMSO) at a concentration of 125 mg mL^{-1} and then plated. The cells were added shortly thereafter and were plated at concentrations of 0.1×10^6 cells mL^{-1} for OVCAR-8 and 0.7×10^5 cells mL^{-1} for HCT-116 and SF-295 lineages. The cells were incubated with the extracts for 72 h in an incubator at 5% CO_2 at 37 °C. Following treatment, cells were washed and fresh medium was prepared. The MTT dye solution (150 µL) was added to each well for 3 h. The absorbance was measured after dissolving the precipitate with 150 µL of pure DMSO on a plate spectrophotometer at 595 nm.[23] Doxorubicin was purchased from Sigma Aldrich (San Diego, USA) and was used as the positive control. Experiments were repeated independently three times. The results are expressed as percentage of cell viability.

Statistical analyses

HCA was used for an initial, exploratory analysis of the cytotoxicity data for the 12 extracts on the three tumor cell line lineages (12 × 3), using preprocessing mean center data and distance to k-nearest neighbor. Then, partial least squares-discriminant analysis (PLS-DA) was used to model the three clusters obtained in the HCA. The data set (12 samples and 110 compound areas) was normalized, and five latent variables were selected to build the model.

Results and Discussion

The extracts obtained by sonication with *n*-hexane, diethyl ether and methanol from the leaves of *S. gardneri, S. macranthera, S. splendida* and *S. trachypus* were submitted to silylation and further analyzed by GC-MS to obtain linear retention indices (RIs) and mass spectra of the compounds, which were compared with the mass spectra in three mass spectra (MS) libraries (i.e., NIST, GMD and Wiley). The RIs were calculated from the retention times obtained from the chromatograms of each compound and of a standard mixture of alkanes (C_{12}-C_{40}), according to Van den Dool and Kratz equation.[24] From the extracts of the four species obtained with the three different solvents, 34 compounds were identified, including carboxylic acids, fatty acids, fatty alcohols, long-chain alkanes, diterpenes, triterpenes and sterols, in addition to the flavonoids chrysin and quercetin. The compounds identified by GC-MS in the extracts of *Senna* species are presented in Table 1.

Each extract was prepared and analyzed in triplicate. With the extracts obtained with the different solvents, the metabolite diversity of the samples was demonstrated. As expected, the more polar extracts obtained with methanol contained high levels of mono-, disaccharides and polyols, whereas the *n*-hexane extracts were rich in terpenoids and fatty acids and other lipophilic compounds. However, the extracts obtained with diethyl ether were less complex. The compounds identified in the GC-MS chemical profiles are shown in Figure 1, and three representative GC-MS chromatograms are shown in Figure 2.

A multivariate analysis using HCA and PLS-DA was conducted of the chemical composition and the cytotoxicity of the extracts against human tumor cells to identify the compounds potentially correlated with this activity. The results of the cytotoxicity assays are shown in Table 2.

The *n*-hexane extract of *S. gardneri* had the highest activity against HTC-116 and SF-295 cancer cell lines with cell growth inhibited by 59.75 and 52.85%, respectively, whereas the highest inhibition of OVCAR-8 cells occurred with the methanol extract of *S. macranthera*. Although the cytotoxicity of plant extracts was generally weak (< 75% inhibition), the cytotoxic potential of these extracts was significantly different. In addition to differences in cytotoxicity, the chemical composition of these extracts was also very different; therefore, we used multivariate analysis of the two data sets (i.e., cytotoxicity and chemical composition) to identify the compounds likely responsible for the biological activity. Thus, the 12 extracts were initially analyzed by HCA, and using cytotoxicity as the dependent variable, the samples were classified into three clusters: (A) the extracts of SgH and SsE with the most activity on HTC-116 and SF-295 cells; (B) the extracts of StM and SmM with the most activity on OVCAR-8 cells; and (C) the extracts with low or no cytotoxicity (Figure 3).

The classification obtained by HCA was then used in a supervised analysis (PLS-DA) to establish a relationship between the extract's cytotoxicity and specific chemical constituents (variables). The PLS-DA was performed using a matrix with 12 cases (extracts) and the areas of chromatographic peaks (12 × 110). The model generated from five latent variables (LV) explained 83.6% of the

Table 1. Compounds and respective peak areas found in the extracts from leaves of *Senna* spp. as trimethylsilyl derivatives in GC-MS analyses

Compound	Rt[a] / min	RI_{exp}[b]	RI_{lit}[c]	MS[d] / %	Compound name	Representative ions (*m/z*)[e]	*Senna* spp. extract[f]
1	6.080	1301	1289	97	succinic acid*	147 (BP)[g], 73, 247	SgM, StM, SsE, SsM, SmM
2	6.268	1314	1336	91	glyceric acid*	73 (BP), 147, 189, 103, 292	SgM, SsM
3	8.615	1397	1404	92	pentanedioic acid*	147 (BP), 73, 261, 158	SsE
4	8.989	1420	1420	93	β-caryophyllene	69 (BP), 93, 133, 79, 91	SgM, SmM
5	10.980	1482	1499	96	malic acid*	73 (BP), 147, 233	SgM, SmH, SmM
6	11.731	1511	1521	96	pyroglutamic acid*	156 (BP), 73, 147	SgM, SmM, SsM
7	14.946	1600	1613	82	3-hydroxy-3-methylglutaric acid*	73 (BP), 103, 147, 219, 247	SsE, SgE
8	21.879	1815	1828	90	benzoic acid, 3,4-dihydroxy*	73 (BP), 193, 370, 355, 311	SgM
9	21.987	1818	1838	92	citric acid*	73 (BP), 193, 370, 355, 311	SgM
10	22.788	1837	1837	94	neophytadiene	66 (BP), 95, 82, 57, 123, 278 [M$^{+\bullet}$][h]	SgE, SgM, StE, StM
11	27.789	1990	1992	91	galactonic acid*	73 (BP), 147, 292, 319, 217	SsE, SsM, StM
12	27.963	1994	1997	92	gluconic acid*	73 (BP), 147, 333, 292, 217	SgH, SgM, SsH, SsE, StH, StM
13	29.571	2046	2047	97	hexadecanoic acid*	117 (BP), 73, 313, 132,145, 328 [M$^{+\bullet}$]	SgH, SgE, SgM, SmH, SmE, SmM, SsH, SsM, SsE, StE, StM
14	31.199	2094	2092	93	linolenic acid methyl ester	79 (BP), 67, 95, 93, 108	SsH, SgH
15	33.242	2162	2164	93	linolenic acid ethyl ester	79 (BP), 67, 95, 93, 108	StE
16	33.393	2172	2183	94	phytol*	143 (BP), 73, 75, 123	SgH, SmH, SsH, SsE, SsM, StH, StM
17	34.403	2207	2212	97	linoleic acid*	73 (BP), 75, 81, 337, 95, 352 [M$^{+\bullet}$]	SgH, SgE, SgM, SmH, SmE, SsH, SsE, SsM, StH, StE, StM
18	34.574	2212	2218	96	α-linolenic acid*	75 (BP), 73, 95, 108, 129, 335, 350 [M$^{+\bullet}$]	SgH, SgE, SgM, SmH, SmE, SsH, SsE, SsM, StH, StE, StM
19	35.459	2243	2248	96	stearic acid*	117 (BP), 73, 341, 132, 129, 356 [M$^{+\bullet}$]	SgH, SgE, SgM, SmH, SmE, SsH, SsE, SsM, StH, StE, StM
20	40.831	2441	2447	92	eicosanoic acid*	117 (BP), 73, 369, 132, 145, 384 (M^{+})	SgH, SmH, StH, StM
21	45.898	2639	2638	92	docosanoic acid*	117 (BP), 73, 397, 132, 145, 412 [M$^{+\bullet}$]	SgH, SmH, SsH, StM
22	46.710	2663	2705	73	chrysin* or isomer	383 (BP), 384, 73	SmE
23	49.998	2807	2812	98	squalene	69 (BP), 81, 121, 137, 410 [M$^{+\bullet}$]	SgH, SgE, SmH, SsH, SsE, StH, StE, StM
24	50.716	2837	2836	90	tetracosanoic acid*	117 (BP), 73, 425, 132, 145, 440 [M$^{+\bullet}$]	SgH, SgE, SsH, SsE, StE, StM
25	53.176	2939	2943	75	*trans*-catechine* or isomer	368 (BP), 73, 283, 578	SmE
26	54.152	2983	3004	76	β-tocoferol*	488 (BP and [M$^{+\bullet}$]), 223, 73	SmH
27	56.925	3120	3149	76	α-tocopherol*	542 (BP and [M$^{+\bullet}$]), 237, 73	SgH, SgE, SmH, SsH, SsE, StE, StM
28	57.501	3164	3171	87	quercetin*	575 (BP), 73, 497	SgH, SmH, SsH, StH, StM
29	59.460	3242	3262	90	stigmasterol*	83 (BP), 129, 255, 394, 484 [M$^{+\bullet}$]	SgH, SgE, SmH, SmM, SsH, SsE, StH, StE, StM
30	60.668	3302	3296	90	β-sitosterol*	129 (BP), 357, 396, 486 [M$^{+\bullet}$]	SgH, SgE, SgM, SmH, SmE, SmM, SsH, SsE, SsM, StH, StE, StM
31	60.895	3314	3312	86	β-amyrin*	218 (BP), 203, 498 [M$^{+\bullet}$]	SmH, SsH, SsE, SsM, StH
32	61.418	3340	3334	96	1-triacontanol*	495 (BP), 75, 97	SgH, SgE, SgM, SmH, SmE, SsH, SsE, SsM, StH, StM
33	61.885	3355	3354	82	α-amyrin*	218 (BP), 189, 498 [M$^{+\bullet}$]	SmH, SsH
34	63.136	3434	3445	–	triacontanoic acid*	117 (BP), 73, 145, 509, 524 [M$^{+\bullet}$]	StH, StM

[a]Rt = retention time; [b]RI_{exp} = experimental retention index; [c]RI_{lit} = retention index from literature; [d]similarity based on NIST MS database; [e]representative ions in decreasing order of abundance; [f]*Senna* ssp.: SgH = *S. gardneri* hexane, SgE = *S. gardneri* ether, SgM = *S. gardneri* methanol, SmH = *S. macranthera* hexane, SmE = *Senna macranthera* ether, SmM = *S. macranthera* methanol, SsH = *S. splendida* hexane, SsE = *S. splendida* ether, SsM = *S. splendida* methanol, StH = *S. trachypus* hexane, StE = *S. trachypus* ether, StM = *S. trachypus* methanol; [g]BP = base peak; [h][M$^{+\bullet}$] = molecular ion; *compounds identified as TMS derivatives.

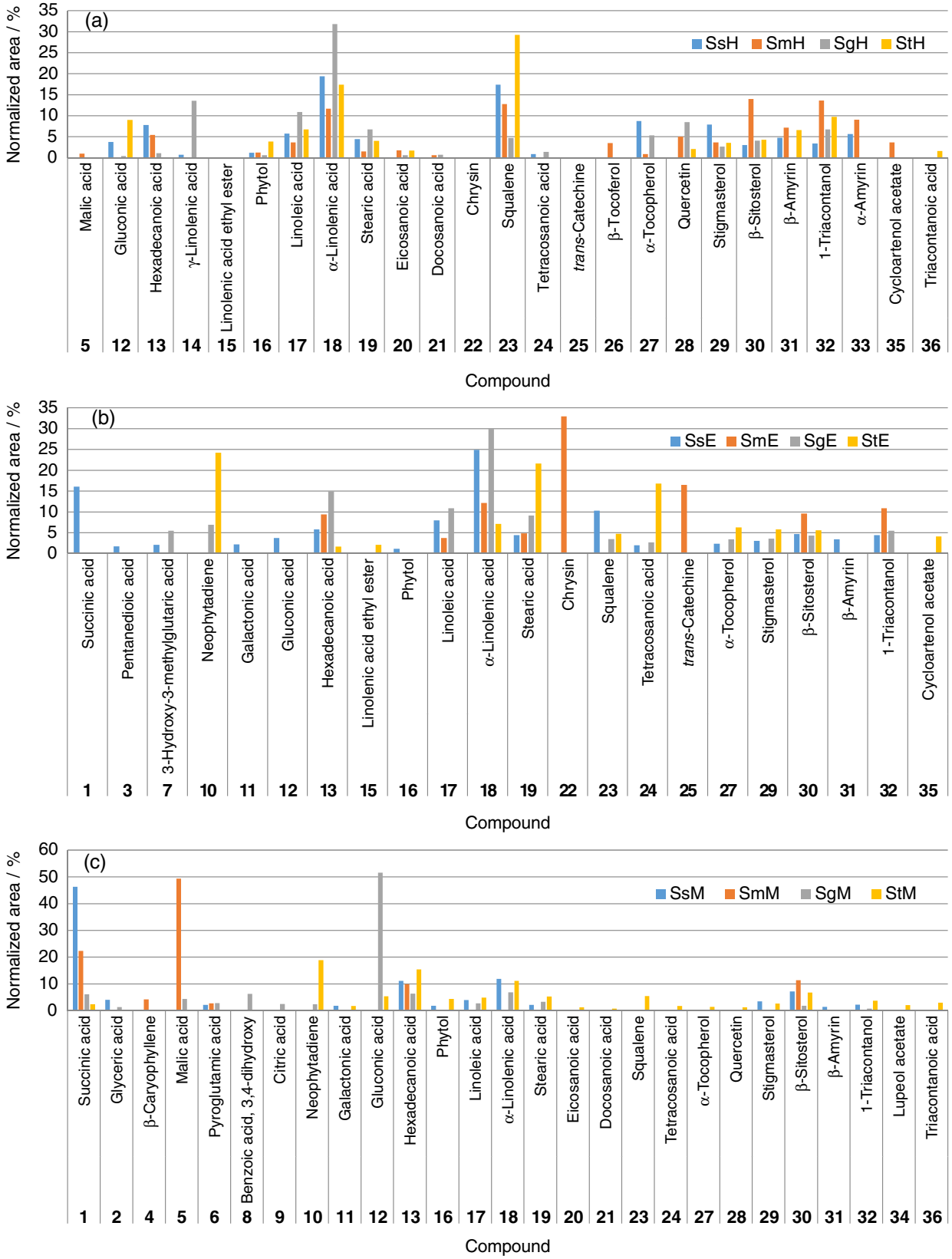

Figure 1. Chemical profiles of *Senna* spp. leaves extracted with (a) hexane; (b) ethyl ether; and (c) methanol. Compounds are shown with more than 1% of the normalized area for each extract. SgH = *S. gardneri* hexane, SgE = *S. gardneri* ether, SgM = *S. gardneri* methanol, SmH = *S. macranthera* hexane, SmE = *Senna macranthera* ether, SmM = *S. macranthera* methanol, SsH = *S. splendida* hexane, SsE = *S. splendida* ether, SsM = *S. splendida* methanol, StH = *S. trachypus* hexane, StE = *S. trachypus* ether, StM = *S. trachypus* methanol extract.

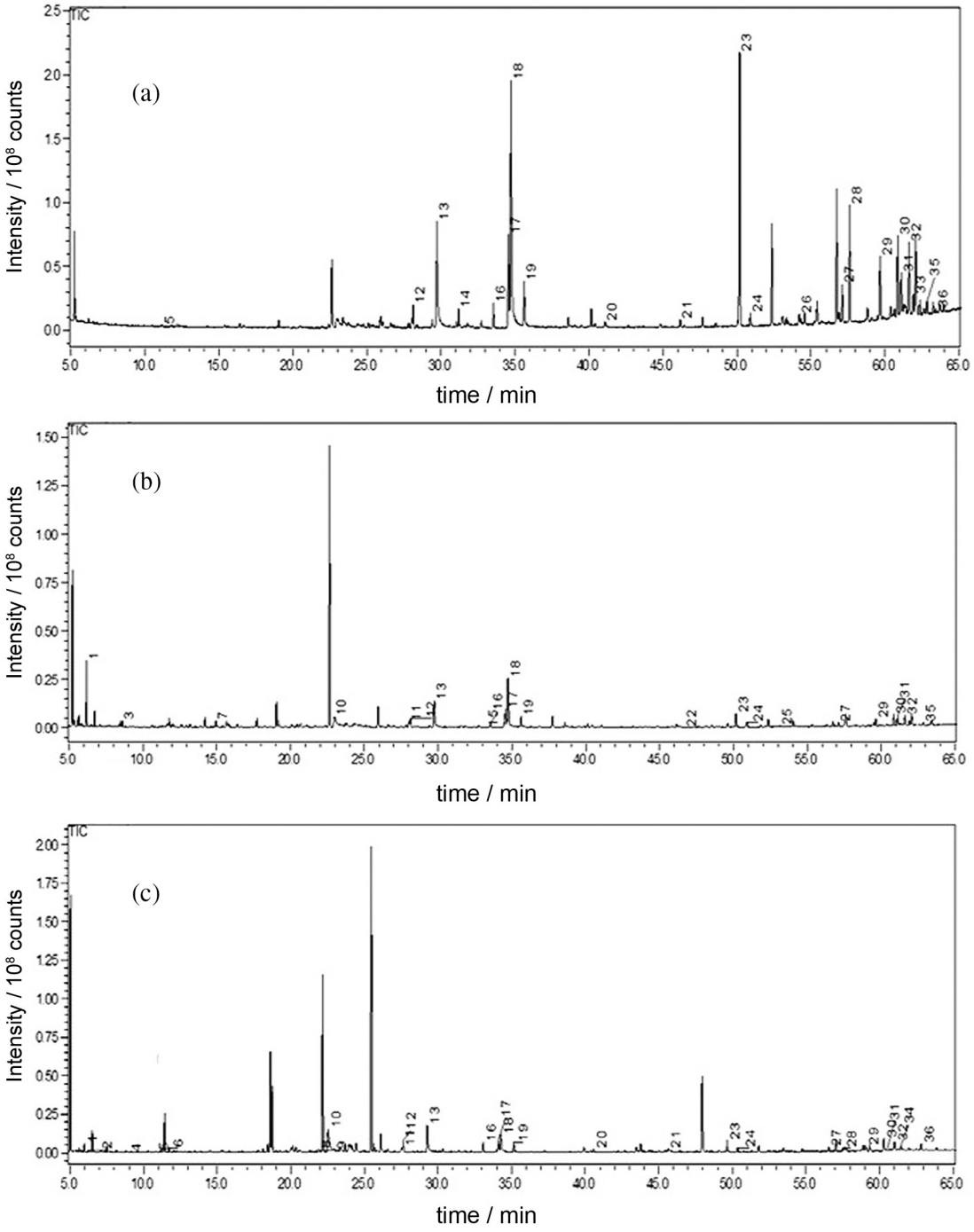

Figure 2. Representative GC-MS chromatograms from (a) hexane extract of leaves from *Senna splendida*; (b) ethyl ether extract of leaves from *Senna splendida*; and (c) methanol extract of leaves from *Senna trachypus.*

variances of the data, with the residual Q and Hotelling's T2 showing only one sample (SgM) outside the 95% confidence intervals. The best adjustments were obtained in the score (Figure 4a) and the loading (Figure 4b) plots of LV4 × LV2 in which the cytotoxic extracts from cluster A (SgH and SsE) were in the identical quadrant (Figure 4a) as variable 53 (Figure 4b), which could be related to the cytotoxicity of these extracts. Variable 53 corresponded to compound **18**, identified as α-linolenic acid (ALA). Several studies examined linoleic acid in combination with other compounds in evaluations of anticancer activity.[25,26]

Dai[27] and Sun *et al.*[28] reported that polyunsaturated fatty acids have inhibitory effects on several tumor cell lines by inducing apoptosis, which corroborates the results obtained with PLS in this study. The ω-3 fatty acids are cytotoxic because of selective anticancer effects through

Table 2. Cytotoxic activity of *Senna* spp. extracts on human cancer cell lines (% inhibition ± SD[a])

Sample[b]	HTC-116[c]	SF-295[d]	OVCAR-8[e]
SgH	59.75 ± 0.60	52.85 ± 5.21	24.61 ± 13.82
SgE	11.63 ± 2.53	24.29 ± 6.13	0.01 ± 0.00
SgM	6.56 ± 10.65	5.77 ± 2.80	0.01 ± 0.00
SmH	28.27 ± 3.05	39.27 ± 7.14	0.01 ± 0.01
SmE	5.59 ± 6.05	20.54 ± 6.22	0.01 ± 0.00
SmM	1.37 ± 3.82	18.56 ± 5.55	44.64 ± 0.99
SsH	13.85 ± 1.29	36.92 ± 0.14	10.43 ± 41.62
SsE	31.37 ± 6.65	48.28 ± 10.04	21.92 ± 1.67
SsM	19.65 ± 5.28	28.66 ± 11.53	0.01 ± 0.00
StH	27.39 ± 3.52	21.19 ± 3.96	0.01 ± 0.00
StE	20.19 ± 1.67	37.12 ± 5.65	9.41 ± 45.94
StM	7.23 ± 1.29	32.38 ± 2.32	35.4 ± 23.31
DOX, IC_{50} [μmol L^{-1}][f]	0.12 (0.09-0.17)	0.22 (0.16-0.24)	0.34 (0.31-0.36)

[a]SD = standard deviation; [b]sample: SgH = *S. gardneri* hexane, SgE = *S. gardneri* ether, SgM = *S. gardneri* methanol, SmH = *S. macranthera* hexane, SmE = *Senna macranthera* ether, SmM = *S. macranthera* methanol, SsH = *S. splendida* hexane, SsE = *S. splendida* ether, SsM = *S. splendida* methanol, StH = *S. trachypus* hexane, StE = *S. trachypus* ether, StM = *S. trachypus* methanol; [c]HTC-116 = human colon; [d]OVACAR-8 = ovarian carcinoma; [e]SF-295 = human glioblastoma; [f]DOX = doxorubicin was the positive control. IC_{50} is the drug concentration that caused 50% inhibition of cell growth, with the corresponding 95% confidence interval (CI 95%) shown below.

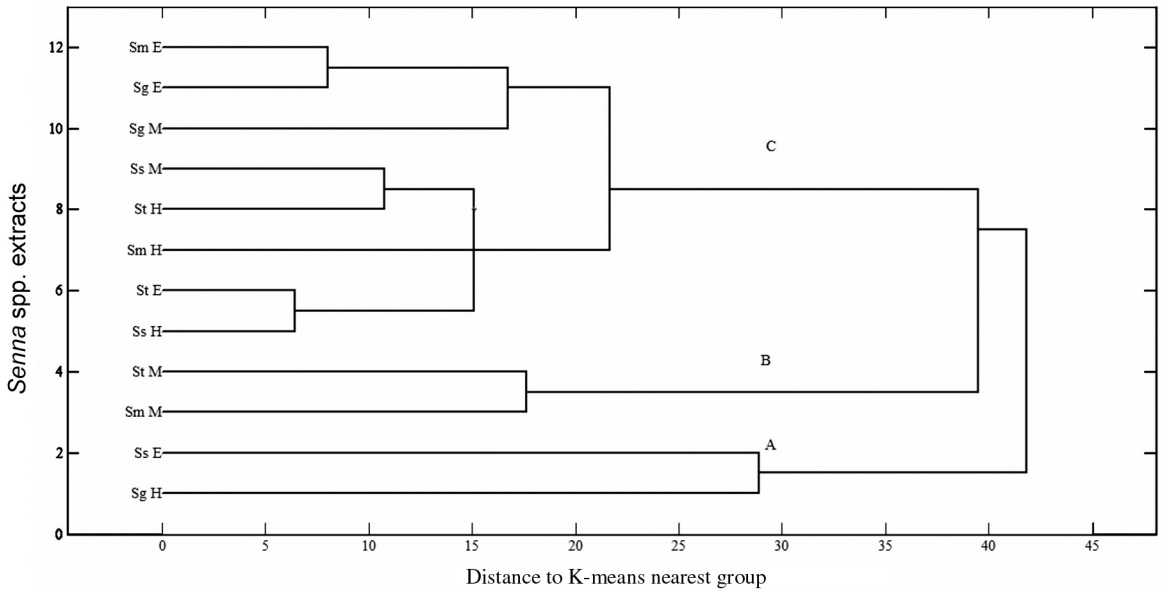

Figure 3. Dendrogram of HCA of the extracts from leaves of *Senna* spp. Cluster A = 2 samples (cytotoxic to HCT-116 and SF-295 cell lines); cluster B = 2 samples (cytotoxic to OVCAR-8 cell line); and cluster C = 8 samples (low and no cytotoxicity).

the generation of free radicals and lipid peroxidation.[29] Dai *et al.*[30] showed that polyunsaturated fatty acids are cytotoxic to tumor cells, and of the fatty acids tested, linoleic acid (LA) and α-linolenic acid (ALA) were the most effective in suppressing the growth of normal gastric cells (GES1) at 180 and 200 μmol L^{-1} and those of gastric carcinoma (MGC and SGC) at 200 μmol L^{-1}. The induction of apoptosis by α-linolenic acid (**18**) (ALA) was observed by Vecchini *et al.*[31] and Scheim[32] and likely occurred because of the reduction of nitric oxide, as proposed by Deshpande *et al.*[33] In recent studies with mouse models, ALA (**18**) reduced breast tumor growth while increasing the efficacy of chemotherapeutic agents. However, these studies did not confirm whether the effects were caused by ALA or its metabolites.[34] No compound was correlated with the cytotoxicity of the samples in cluster B, which included two methanolic extracts, most likely because several of the polar compounds in these extracts did not elute in the chromatographic conditions used in this study.

Additionally, some compounds were detected in only one species; for example, the flavonoid, chrysin (**22**), was identified only in *S. macranthera*, and the flavonoid,

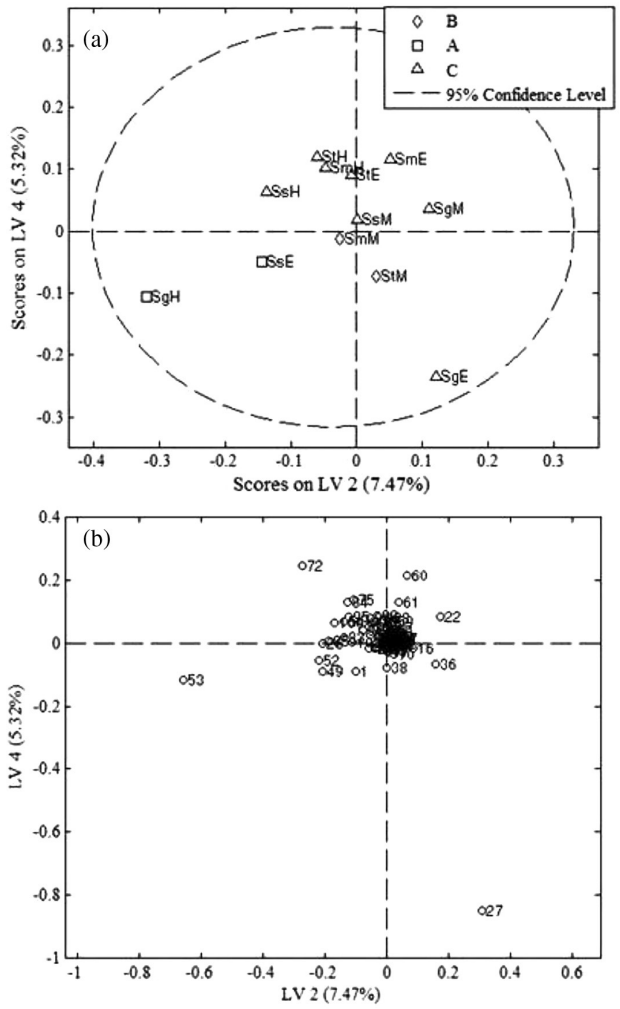

Figure 4. Score (a) and loading (b) plots obtained after PLS-DA using 12 extracts from *Senna* spp. and 110 compound areas (12 × 110). A) extracts cytotoxic to HTC-116 and SF-295 cells; B) extracts cytotoxic to OVCAR-8 cells; and C) extracts with low or no cytotoxicity.

quercetin (**28**), was identified only in the methanolic extract of *S. trachypus*. These compounds have shown excellent potential for chemopreventive and cancer therapy[35] and have anti-inflammatory and antioxidant activities.[36] However, eleven compounds were identified in all the extracts that are typically encountered in different families and genera of higher plants. For example, all extracts contained the triterpene squalene (**23**). This compound, which is produced by all higher organisms and has beneficial effects on human health and antioxidant activity, has also been isolated from the leaves and roots of *Ramonda serbica* and *R. nathaliae*,[37] and from the marine diatom *Pleurosigma strigosum*.[38] The triterpenes α-amyrin (**33**) and β-amyrin (**31**) were detected only in the *n*-hexane extracts of leaves of *S. macranthera* and *S. splendida*. These compounds, with anti-inflammatory, anti-conceptive, and hepatoprotective pharmacological activities, have also been isolated as a mixture from different natural sources, including other species of the genus *Senna*, i.e., *S. spectabilis* var. *excelsa* and *S. reticulata*.[7] The steroids β-sitosterol (**30**) and stigmasterol (**29**) are common compounds in species of several genera and families, and the properties of β-sitosterol isolated from *S. spectabilis* var. *excelsa* have been reported as antibacterial, anti-inflammatory and analgesic.[6]

Conclusions

The GC-MS analyses of 12 extracts from the leaves of four species of *Senna* identified 34 compounds in different groups that included fatty acids, steroids, triterpenes and flavonoids not reported previously for these species. The use of multivariate analyses (HCA and PLS-DA) led us to infer that the cytotoxicity of some *Senna* extracts to HTC-116 and SF-295 tumor cells lines was attributed to linolenic acid, an inference that was reinforced by literature data. Moreover, with analytical techniques, dereplication and multivariate statistical analysis, this study demonstrated that it was possible to effectively identify hit compounds and by avoiding the steps of extract fractionation and purification of known compounds, to improve the research approach in the prospect for new drug prototypes from biological sources.

Supplementary Information

Supplementary data (MS spectra) are available free of charge at http://jbcs.sbq.org.br.

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