Evaluation of the Cytotoxic Activity of Some Brazilian Medicinal Plants

Authors Sandra S. Ribeiro[1], Aline M. de Jesus[1], Charlene S. dos Anjos[1], Thanany B. da Silva[1], Alan D. C. Santos[1], Jemmyson R. de Jesus[1], Moacir S. Andrade[1], Tais S. Sampaio[1], Wesley F. Gomes[1], Péricles B. Alves[1], Adriana A. Carvalho[2], Claudia Pessoa[2], Manoel O. de Moraes[2], Maria L. B. Pinheiro[3], Ana Paula N. Prata[4], Arie F. Blank[5], Renata Silva-Mann[5], Valeria R. S. Moraes[1], Emmanoel V. Costa[1], Paulo Cesar L. Nogueira[1], Daniel P. Bezerra[6]

Affiliations The affiliations are listed at the end of the article

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- *Guatteria hispida*
- *Jatropha curcas*
- *Kielmeyera rugosa*
- *Lippia gracilis*
- extracts
- essential oil

Abstract

Plants are promising sources of new bioactive compounds. The aim of this study was to investigate the cytotoxic potential of nine plants found in Brazil. The species studied were: *Annona pickelii* Diels (Annonaceae), *Annona salzmannii* A. DC. (Annonaceae), *Guatteria blepharophylla* Mart. (Annonaceae), *Guatteria hispida* (R.E. Fr.) Erkens & Maas (Annonaceae), *Hancornia speciosa* Gomes (Apocynaceae), *Jatropha curcas* L. (Euphorbiaceae), *Kielmeyera rugosa* Choisy (Clusiaceae), *Lippia gracilis* Schauer (Verbenaceae), and *Hyptis calida* Mart. Ex Benth (Lamiaceae). Different types of extractions from several parts of plants resulted in 43 extracts. Their cytotoxicity was tested against HCT-8 (colon carcinoma), MDA-MB-435 (melanoma), SF-295 (glioblastoma), and HL-60 (promielocitic leukemia) human tumor cell lines, using the thiazolyl blue test (MTT) assay. The active extracts were those obtained from *G. blepharophylla, G. hispida, J. curcas, K. rugosa*, and *L. gracilis*. In addition, seven compounds isolated from the active extracts were tested; among them, *β*-pinene found in *G. hispida* and one coumarin isolated from *K. rugora* showed weak cytotoxic activity. In summary, this manuscript contributes to the understanding of the potentialities of Brazilian plants as sources of new anticancer drugs.

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Correspondence
Prof. Dr. Daniel P. Bezerra
Department of Physiology
Federal University of Sergipe
Av. Marechal Rondon, Jardim Rosa Elze
49100-000 São Cristóvão
Sergipe
Brazil
Phone: + 55 79 21 05 66 44
danielpbezerra@gmail.com

Introduction

Nature is an important source of new candidates for therapeutic compounds, as a large chemical diversity is found in several species of plants, animals, and microorganisms. In many cases, this chemical diversity reflects self-defense mechanisms that represent the strategies employed to repel or destroy predators [1].

Many plant-derived compounds are currently successfully employed in cancer chemotherapy. In fact, the importance of plants as a source of new anticancer agents has been emphasized by several researchers [2–8]. An analysis of the number of chemotherapeutic agents and their sources indicates that over 50% of approved drugs are derived from natural compounds [9].

One of the most significant examples is the vinca alkaloid family isolated from *Catharanthus roseus* (L.) G. Don. The introduction of the vinca alkaloid vincristine was responsible for an increase in the cure rates for Hodgkin's disease and some forms of leukemia [10, 11]. Another example of a highly active agent derived from a natural product is etoposide, which has produced high cure rates in testicular cancer when used in combination with bleomycin and cisplatin. Etoposide inhibits the enzyme topoisomerase II, which unwinds DNA, and by doing so causes DNA strands to break [12]. The taxanes paclitaxel and docetaxel, also derived from a natural product, show antitumor activity against breast, ovarian, and other tumor types in the clinic. Like vinca alkaloids, taxanes are anti-mitotic and anti-microtubule agents [13, 14].

The present study was designed to investigate the cytotoxic potential of nine plants found in Brazil against tumor cell lines. The species studied were: *Annona pickelii* Diels (Annonaceae), *Annona salzmannii* A. DC. (Annonaceae), *Guatteria blepharophylla* Mart. (Annonaceae), *Guatteria hispida* (R.E. Fr.) Erkens & Maas (Annonaceae), *Hancornia speciosa* Gomes (Apocynaceae), *Jatropha curcas* L. (Euphorbiaceae), *Kielmeyera rugosa* Choisy (Clusiaceae), *Lippia gracilis* Schauer (Verbenaceae), and *Hyptis calida* Mart. Ex Benth (Lamiaceae).

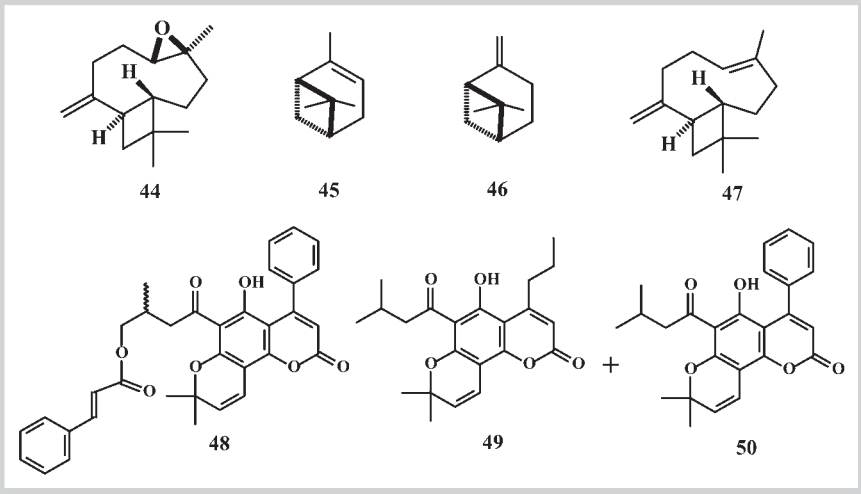

Fig. 1 Chemical structure of caryophyllene oxide (**44**), α-pinene (**45**), β-pinene (**46**), (*E*)-caryophyllene (**47**), KRS6 (**48**), and KRS7 (**49 + 50**).

The supplementary **Table 1S** shows their medicinal use. Additionally, seven compounds isolated (**Fig. 1**) from active extracts were also tested.

Materials and Methods

Reagents

Methanol, hexane, petroleum ether, dichloromethane, ethyl acetate, chloroform, and dimethyl sulfoxide (DMSO) were purchased from F. Maia or Vetec Química Fina Ltd. Glutamine, RPMI 1640 medium, fetal bovine serum, penicillin, and streptomycin were obtained from Cultilab. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and doxorubicin (purity > 98 %) were obtained from Sigma-Aldrich. Carbon dioxide (CO_2) was purchased from White Martins.

Plant material

Leaves and bark of *A. salzmannii* (voucher number 15438) and leaves and branches of *A. pickelii* (voucher number 15439) were collected in March 2010 in "Mata do Crasto", Municipality of Santa Luzia do Itanhy, Sergipe State, Brazil, at coordinates [S 11°23′ 12″ W 037°25′05″] and [S 11°23′01″ W 037°25′13″], respectively. Leaves, branches, fruit, and latex from the fruit of *H. speciosa* (voucher number 13630) were collected from orchards located in Sergipe State, Brazil, between October 2002 and June 2003. Leaves of *J. curcas* (voucher number JC014URVES) were collected in January 2009 (for fresh leaf analysis) and March 2009 (for air-dried leaf analysis) from the Germplasm Bank of the Department of Agronomic Engineering at the Federal University of Sergipe in the Municipality of São Cristóvão, Sergipe, Brazil. Leaves of the accession named EMB, from a *J. curcas* specimen, were collected in October 2008 (for fresh and air-dried leaf analysis) from the experimental campus of "Embrapa Tabuleiros Costeiros", in the Municipality of Umbaúba, Sergipe, Brazil. Leaves, stems, and fruit of *K. rugosa* (voucher number 206) were collected from a "restinga" (the vegetation mosaic found in Brazilian coastal sandy plains) near the Pomonga River in the Municipality of Santo Amaro das Brotas, Sergipe, Brazil. Leaves and stems of the accession named FUS, from an *L. gracilis* specimen (voucher number 9205) were collected in May 2007 from the campus of the Federal University of Sergipe in the Municipality of São Cristóvão, Sergipe, Brazil. Leaves from another specimen of *L. gracilis* (voucher number 18740) and leaves of *H. calida* (voucher number 18741) were harvested in November 2006 in the Municipality of Poço Redondo, Sergipe, Brazil. All leaves were obtained from flowering species. The species were identified by Dr. Ana Paula do Nascimento Prata, a plant taxonomist from the Department of Biology at the Federal University of Sergipe (UFS). The voucher botanic specimens are deposited at the Herbarium of the Federal University of Sergipe.

Leaves of *G. blepharophylla* (voucher number 7340) were collected in January 2005 at the Federal University of Amazonas (UFAM), Manaus, Amazonas, Brazil. The leaves of *G. hispida* (voucher number 7707) were collected in February 2005 at the Adolpho Ducke Reserve in the vicinity of Manaus, Amazonas, Brazil. All leaves were obtained from flowering species. The species were identified by Annonaceae specialist Dr. Antonio Carlos Webber from the Department of Biology at the Federal University of Amazonas (UFAM). The voucher botanic specimens are deposited at the Herbarium of the Federal University of Amazonas.

Plant extractions

The dried, powdered bark (1800 g) of *A. salzmannii* was submitted to exhaustive extractions with 5 × 4 L of hexane and methanol to give hexane (8.69 g) and methanol (143.29 g) extracts after removal of each solvent, while the dried, powdered leaves (614 g) were submitted to exhaustive extractions with 5 × 1.5 L of petroleum ether and methanol to give petroleum ether (40.28 g) and methanol (78.74 g) extracts after removal of each solvent.

The dried, powdered branches (330 g) and leaves (363 g) of *A. pickelii* were submitted to exhaustive extractions with 5 × 1 L of petroleum ether and methanol to give petroleum ether (1.88 g and 14.54 g) and methanol (16.12 g and 42.16 g) extracts after removal of each solvent, respectively.

The fresh, air-dried (for five days) leaves from *J. curcas* were separately triturated and extracted by maceration at room temperature with methanol (1 : 4 v/v) over five days. The solutions obtained were filtrated through analytical filter paper and the solvent removed under reduced pressure to give the correspondent crude extracts of fresh and air-dried leaves. The extraction steps for each sample were repeated two times.

The extracts and partitions of *K. rugosa* were obtained according to the method described by Nogueira et al. [15]. The air-dried, powdered leaves (163.4 g) and stems (397.4 g) of *K. rugosa* were extracted at room temperature with methanol. The solvent was

removed under reduced pressure to give the correspondent crude extracts of leaves (31.5 g) and stems (20.9 g). The stems were also subjected to maceration with dichloromethane during 48 hours. After this period, the solvent was removed under reduced pressure to give dichloromethane extract (90.3 g). The CH_2Cl_2 extract was suspended in MeOH : H_2O (1 : 1) solution and extracted successively with petroleum ether (2.1 g), CH_2Cl_2 (20.6 g), and EtOAc (5.3 g).

The extracts of *L. gracilis* were obtained according to the method described by Gomes et al. [16, 17]. The air-dried, powdered leaves (54.0 g) of *L. gracilis* (a specimen harvested in Poço Redondo, Sergipe, Brazil) was submitted to exhaustive extractions with hexane and methanol to give hexane (3.0 g) and methanol (11.9 g) extracts after removal of each solvent, while the air-dried, powdered leaves (180.4 g) and stems (577.1 g) from another specimen collected in the campus of the Federal University of Sergipe (the accession named FUS) were extracted separately at room temperature with methanol (1 : 5 w/v) by maceration during a week. The solution obtained was filtrated through analytical filter paper and the solvent removed under reduced pressure to give the correspondent crude extracts of leaves (12.2 g) and stems (7.8 g).

The dried branches, fruit, and leaves of *H. speciosa* and the dried leaves of *H. calida* were triturated and extracted by maceration at room temperature with hexane and methanol. The solutions obtained were filtrated through analytical filter paper and the solvent removed under reduced pressure to give the corresponding crude extracts. The extraction steps for each sample were repeated two times. Part of each methanol extract of *H. speciosa* was suspended in MeOH : H_2O (9 : 1) solution and extracted successively with hexane, $CHCl_3$, and EtOAc to give the corresponding partitioned extracts. Unripe fresh fruit from *H. Speciosa* was cut, the latex obtained was extracted with CH_2Cl_2, and the solvent was removed under reduced pressure to give the corresponding crude extract.

The essential oils from *A. salzmannii, A. pickelii, G. blepharophylla, G. hispida* (200 g each, dried leaves), and *L. gracilis* (50 g each, fresh leaves) were obtained by hydrodistillation for 3 h, using a Clevenger-type apparatus (Amitel). The essential oils were dried over anhydrous sodium sulphate, and the percentage content was calculated on the basis of the dry weight of plant material. The essential oils were stored in a freezer until further analysis. The extractions of the oils were performed in duplicate for Annonaceae species and in triplicate for *L. gracilis*.

Pure compounds

Caryophyllene oxide (**44**, purity 99%) was obtained from the essential oil of the leaves of *G. blepharophylla*, and its structure was established based on spectroscopic studies [18]. α-Pinene (**45**, purity ≥ 98%), β-pinene (**46**, purity ≥ 97%), and (*E*)-caryophyllene (**47**, purity ≥ 86%) present in the essential oil of *G. hispida* [18] were obtained from Aldrich Chemical Company.

The coumarins KRS6 (**48**, 5-hydroxy-6-(4-cinnamoyl-3-methyl-1-oxobutyl)-4-phenyl-6′,6′-dimethylpyrano (2′,3′:7,8)-coumarin, purity 83.3%) and KRS7 (**49**, 5-hydroxy-6-(3-methyl-1-oxobutyl)-4-*n*-propyl-6′,6′-dimethylpyrano (2′,3′:7,8)-coumarin + **50**, 5-hydroxy-6-(2-methyl-1-oxobutyl)-4-*n*-propyl-6′,6′-dimethylpyrano (2′,3′:7,8)-coumarin, purity 71.3%) were isolated from the dichloromethane extract from stems of *K. rugosa*, according to the method described by Nogueira et al. [15]. A part of the dichloromethane extract from stems (4.0 g) was subjected to successive silica gel column eluting with hexane, CH_2Cl_2, EtOAc, and MeOH as binary mixtures of increasing polarity to yield compound **48** (9.6 mg, yellowish amorphous solid) and a mixture of compounds **49** and **50** (7.0 mg, yellowish oil).

Cytotoxicity assay

The cytotoxicity of the extracts was tested against HCT-8 (colon carcinoma), MDA-MB-435 (melanoma), SF-295 (glioblastoma), and HL-60 (promielocitic leukemia) human tumor cell lines (American National Cancer Institute). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 µg/mL streptomycin, and 100 U/mL penicillin at 37 °C with 5% CO_2. For the experiments, the cells were placed in 96-well plates (0.7×10^5 cells/mL for adherent cells or 0.3×10^6 cells/mL for suspended cells in 100 µL of medium). After 24 h, the extracts, dissolved in DMSO (1%) at a final concentration of 50 µg/mL, were added to each well and incubated for 3 days (72 h). Tumor cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product [19]. At the end of 72 h incubation, the medium in each well was replaced by fresh medium (200 µL) containing 0.5 mg/mL of MTT. Three hours later, the formazan product from the MTT reduction was dissolved in DMSO (150 µL), and the absorbance was measured using a multiplate reader (DTX 880 Multimode Detector; Beckman Coulter Inc.). Extract effect was quantified as the percentage of control absorbance of reduced dye at 595 nm.

The experiment was performed with three replicates per treatment, using DMSO at 1% and doxorubicin at 100 µg/mL as negative and positive controls, respectively. All absorbance values were converted into a cell growth inhibition percentage (GI%) by the following formula:

$$GI\% = 100 - [(T/C) \times 100\%]$$

C was the absorbance for the negative control, and *T* was the absorbance in the presence of the tested extract. Those extracts that caused more than 75% cell growth inhibition in any cell line were tested again at concentrations varying from 0.024 to 50 µg/mL to determine the 50% inhibitory concentration (IC_{50}). Extracts with an IC_{50} value lower than 30 µg/mL were considered promising for the search for new anticancer agents [20].

Statistical analysis

Data are presented as the mean ± SD. The IC_{50} values were obtained by nonlinear regression using the GraphPad program (Intuitive Software for Science).

Supporting Information

Table 1S shows the medicinal use of the investigated plants.

Results and Discussion

The role of natural products as a source for remedies has been recognized since ancient times. Drugs derived from natural products are offering us a great opportunity to evaluate both new chemical classes of therapeutic agents and novel mechanisms of action [1]. Brazil has one of the largest biodiversities in the world, and several of these plants have been used to treat a large number of diseases, including cancer [21–23]. Based on this context, in this work we evaluated the cytotoxic potential of nine

Table 1 List of plants and their growth inhibitory effects against tumor cell lines.

No.	Plant species	Plant parts[a] (Extractions[b])	Cell[c] growth inhibition percentage (GI%)[d]		
			MDA-MB-435	SF-295	HCT-8
1	*Annona pickelii* Diels	L (pe)	38.9 ± 1.1	Nd	62.6 ± 1.3
2		L (m)	30.8 ± 3.5	Nd	52.5 ± 3.7
3		L (eo)	51.0 ± 2.2	Nd	64.4 ± 2.9
4		BC (m)	9.9 ± 3.1	Nd	35.8 ± 4.5
5		BC (pe)	30.9 ± 4.8	Nd	60.1 ± 2.6
6	*Annona salzmannii* A. DC.	L (eo)	68.0 ± 5.3	Nd	89.2 ± 6.6
7		L (pe)	46.9 ± 1.7	Nd	64.5 ± 0.8
8		L (m)	34.7 ± 3.3	Nd	49.5 ± 0.9
9		B (h)	52.7 ± 0.2	Nd	62.2 ± 2.1
10		B (m)	56.6 ± 7.2	Nd	61.2 ± 4.6
11	*Guatteria blepharophylla* Mart.	L (eo)	99.7 ± 0.8	Nd	100.0 ± 0.2
12	*Guatteria hispida* (R. E. Fr.) Erkens & Maas	L (eo)	100.0 ± 0.2	Nd	100.0 ± 0.1
13	*Hancornia speciosa* Gomes	LF (dcm)	12.2 ± 1.6	25.8 ± 7.9	1.3 ± 1.5
14		L (m)	17.4 ± 6.5	16.8 ± 4.8	25.6 ± 8.5
15		L (h)	19.6 ± 0.9	29.7 ± 8.1	10.3 ± 10.3
16		L (m/ea)	19.6 ± 9.5	22.7 ± 1.3	25.8 ± 5.8
17		L (m/c)	24.4 ± 4.4	20.5 ± 1.4	25.2 ± 4.0
18		BC (m)	0.0	15.7 ± 6.1	18.6 ± 8.9
19		BC (h)	10.9 ± 7.2	23.7 ± 8.9	2.7 ± 6.6
20		BC (m/c)	0.0	24.3 ± 9.1	31.8 ± 2.5
21		DRF (m)	18.9 ± 6.2	13.5 ± 1.5	12.2 ± 1.0
22		DRF (m/c)	12.9 ± 4.0	3.9 ± 0.3	33.6 ± 4.6
23		UFL (m)	19.0 ± 8.2	20.1 ± 1.0	14.3 ± 0.5
24		RFHP (m)	21.3 ± 8.9	24.2 ± 0.1	4.5 ± 2.4
25		FDL (m)	24.5 ± 9.2	28.1 ± 0.3	23.2 ± 2.5
26	*Jatropha curcas* L.	DL (m)	48.3 ± 1.9	Nd	40.4 ± 5.8
27		FL (m)	80.7 ± 7.4	55.0 ± 6.6	88.3 ± 0.3
28		FEMB (m)	41.3 ± 2.3	37.2 ± 3.2	100.0 ± 0.3
29		DEMB (m)	15.1 ± 2.3	11.2 ± 7.7	24.6 ± 0.6
30	*Kielmeyera rugosa* Choisy	S (m)	33.2 ± 3.2	45.2 ± 4.0	22.0 ± 0.3
31		S (dcm/pe)	72.8 ± 5.9	92.3 ± 0.4	80.3 ± 2.3
32		S (dcm)	23.0 ± 3.1	21.3 ± 8.5	20.4 ± 7.9
33		S (dcm/ea)	8.6 ± 4.4	22.9 ± 6.3	20.5 ± 4.0
34		S (dcm/dcm)	43.2 ± 8.1	67.9 ± 12.4	79.0 ± 0.5
35		L (m)	38.8 ± 5.0	49.9 ± 3.2	37.9 ± 3.6
36	*Lippia gracilis* Schauer	L (h)	0.2 ± 1.7	14.6 ± 1.1	7.3 ± 3.1
37		L (m)	41.2 ± 3.7	20.3 ± 4.1	7.3 ± 3.1
38		L (eo)	94.9 ± 5.8	Nd	95.1 ± 0.8
39		SFUS (m)	5.4 ± 0.8	17.9 ± 5.5	15.0 ± 3.0
40					
41		LFUS (m)	0.0	13.6 ± 4.0	58.3 ± 5.8
42	*Hyptis calida* Mart. Ex Benth.	L (h)	0.0	19.3 ± 1.2	8.7 ± 5.3
43		L (m)	0.0	7.2 ± 3.2	8.7 ± 5.3
	Doxorubicin[e]		100.0 ± 0.3	98.2 ± 0.5	100.0 ± 0.1

[a] Plant parts: L, leaves; B, bark; S, stem; BC, branches; F, flowers; LF, latex from fruit; DRF, dried ripe fruit; UFL, unripe fruit latex-free; RFHP, dried ripe fruit after headspace with Porapak; FDL, green fruit with latex; DL, dried leaves; FL, fresh leaves; FEMB, fresh leaves from accession EMB; DEMB, dried leaves from accession EMB; LFUS, leaves from accession FUS; SFUS, stem from accession FUS. [b] Extractions: h, hexane; m, methanol; pe, petroleum ether; eo, essential oil; dcm, dichloromethane; dcm/ea, dichloromethane extract-ethyl acetate partition; dcm/pe, dichloromethane extract-petroleum ether partition; dcm/dcm, dichloromethane extract-dichloromethane partition; m/ea, methanol extract-ethyl acetate partition; m/c, methanol extract-chloroform partition. [c] Cell lines: MDA-MB-435 (human melanoma), SF-295 (human glioblastoma), and HCT-8 (human colon carcinoma). [d] GI% values are presented as the mean ± SD from three replicates measured by the MTT assay after 72 hours of incubation. All extracts were tested at a concentration of 50 µg/mL. [e] Doxorubicin was used as the positive control. Nd: not determined

plants belonging to 5 families found in Brazil. The cytotoxicity was assessed against four human tumor cell lines (HCT-8, MDA-MB-435, SF-295, and HL-60), using the thiazolyl blue test (MTT) assay. MTT assay is a well-characterized colorimetric assay that is based on the enzymatic reduction of the tetrazolium salt MTT in living, metabolically active cells, but not in dead cells. It has been largely used to determine cytostatic/cytotoxic potential of medicinal agents in screening programs [5–7].

Different types of extractions from several parts of plants resulted in 43 extracts. The plant species and the plant parts used for extract preparation are shown in **Table 1**. The cytotoxicity of the extracts was initially tested against tumor cell lines using the thiazolyl blue test (MTT) assay at a concentration of 50 µg/mL (**Table 1**). Those extracts that caused more than 75% cell growth inhibition in any cell line were considered active. Additionally, concentration-response curves were generated, and IC_{50} values were calculated for these active extracts (**Table 2**).

Table 2 IC_{50} values of plant extracts and isolated compounds against tumor cell lines.

No.	Plant species	Plant parts[a] (Extractions[b])	Cell line[c] (IC_{50})[d]			
			HL-60	MDA-MB-435	SF-295	HCT-8
1	*Annona salzmannii* A. DC.	L (eo)	> 50	46.6 ± 4.2	> 50	48.3 ± 4.1
11	*Guatteria blepharophylla* Mart.	L (eo)	3.3 ± 0.3	22.2 ± 1.7	24.3 ± 2.1	13.1 ± 2.4
44	Caryophyllene oxide		> 25	> 25	> 25	> 25
12	*Guatteria hispida* (R. E. Fr.) Erkens & Maas	L (eo)	1.6 ± 0.3	14.0 ± 0.2	5.7 ± 0.2	6.5 ± 0.2
45	α-Pinene		> 25	> 25	> 25	> 25
46	β-Pinene		> 25	> 25	26.3 ± 0.8	24.1 ± 0.4
47	(*E*)-Caryophyllene		> 25	> 25	> 25	> 25
26	*Jatropha curcas* L.	DL (m)	30.8 ± 0.2	63.7 ± 0.3	> 50	48.2 ± 0.2
28		FEMB (m)	25.1 ± 0.2	53.4 ± 0.2	> 50	48.3 ± 0.3
31	*Kielmeyera rugosa* Choisy	S (dcm/pe)	7.4 ± 0.4	12.6 ± 0.3	3.5 ± 0.2	1.4 ± 0.2
34		S (dcm/dcm)	> 50	54.6 ± 0.1	> 50	65.4 ± 0.2
48	KRS6		> 25	> 25	> 25	23.2 ± 0.2
49 + 50	KRS7		> 25	> 25	> 25	> 25
38	*Lippia gracilis* Schauer	L (eo)	3.6 ± 0.1	3.1 ± 0.1	6.1 ± 0.2	3.1 ± 0.2
	Doxorubicin[e]		0.02 ± 0.10	0.5 ± 0.2	0.2 ± 0.1	0.04 ± 0.10

[a] Plant parts: L, leaves; S, stem; DL, dried leaves; FEMB, fresh leaves from accession EMB. [b] Extractions: m, methanol; eo, essential oil; dcm/pe, dichloromethane extract-petroleum ether partition; dcm/dcm, dichloromethane extract-dichloromethane partition. [c] Cell lines: HL-60 (human leukemia), DA-MB-435 (human melanoma), SF-295 (human glioblastoma), and HCT-8 (human colon carcinoma). [d] Data are presented as IC_{50} values ± SD (μg/mL) from two independent experiments performed in duplicate, measured by the MTT assay after 72 hours of incubation. [e] Doxorubicin was used as the positive control

According to Suffness and Pezzuto [20], only those extracts presenting IC_{50} values below 30 μg/mL in tumor cell line assays are considered promising for anticancer drug development. Thus, only those extracts obtained from *G. blepharophylla, G. hispida, J. curcas, K. rugosa*, and *L. gracilis* presented promising results based on the IC_{50} values (**Table 2**).

One of the most potent cytotoxic activities was found for the dichloromethane extract-petroleum ether partition from stems of *K. rugosa*, with IC_{50} values ranging from 1.4 to 12.6 μg/mL. Additionally, three coumarins isolated from *K. rugosa* were tested, KRS6 (**48**) and KRS7 (**49 + 50**); however, only KRS6 (**48**) showed weak cytotoxic activity (**Table 2**). Phytochemical studies of *K. rugosa* also include the isolation of lupeol and α-amyrin, which are described as cytotoxic agents [15, 24, 25]. In the Brazilian *Kielmeyera* species, xanthones and 4-alkyl and 4-phenyl coumarins have been mainly reported [15]. Plant parts, especially the leaves of these species, have been frequently used in folk medicine to treat several tropical diseases, including schistosomiasis, leishmaniasis, and malaria, as well as bacterial and fungal infections [26].

The anticancer potential of *J. curcas* has been reported. Extracts from *J. curcas* have found a number of traditional medical uses and have been intensively investigated for their secondary metabolites [27, 28]. A potent activity was exhibited by a large number of these compounds, including curcusone A, curcusone B, curcusone C, curcusone D, curcin, ultidione, jatropholone, and acetoxyjatropholone. The mechanism(s) of their antiproliferative activity has not been investigated; however, the presence of Michael acceptors, in some cases, could be associated to cytotoxic activities [29]. Additionally, curcusone B also effectively suppresses the metastatic processes at doses that are nontoxic to cells, which may be of therapeutic benefit for the treatment of metastatic cancers [30].

A toxic protein was also isolated from the seeds of *J. curcas,* curcin, which is a type of ribosome-inactivating protein. Different effects of curcin on various tumor cells were observed, with SGC-7901 (gastric cancer cell line), Sp2/0 (mouse myeloma cell line), and human hepatoma being the most sensitive to curcin, and Hela (carcinoma cell line) being the most resistant to curcin. Its mechanisms are related to the *N*-glycosidase activity [31].

Those extractions (essential oils) obtained from *G. blepharophylla, G. hispida*, and *L. gracilis* also presented strong cytotoxic activity. The main compound found in the leaf oil of *G. blepharophylla* is caryophyllene oxide (**44**) (69%). The major constituents identified in the leaf of *G. hispida* are β-pinene (**46**) (38%), α-pinene (**45**) (31%), and (*E*)-caryophyllene (**47**) (21%) [18]. To investigate whether these compounds are responsible for the cytotoxic activity of the extracts, their cytotoxic activities were also tested; however, only β-pinene (**46**) showed weak cytotoxic activity (**Table 2**). It seems therefore that associations of compounds or the minor compounds are responsible for their cytotoxic activity.

The anticancer potential of extracts and isolated compounds from the bark of *G. blepharophylla* has been reported. The *n*-hexane and MeOH crude extracts and the isolated compound, liriodenine, showed significant activity against cell lines [32]. On the other hand, the cytotoxic potential of the essential oils obtained from *G. blepharophylla, G. hispida*, and *L. gracilis* were reported here for the first time.

Those extracts obtained from *A. pickelii, A. salzmannii, H. speciosa, L. gracilis,* and *H. calida* did not show any expressive antiproliferative effect. The cytotoxicity of the essential oils of *A. pickelii* and *A. salzmannii* were previously investigated [33]. They presented weak cytotoxic effects (cytotoxicity in concentrations higher than 30 μg/mL). In addition, three isolated compounds (cyclitols quinic acid, l-(+)-bornesitol, and rutin) from the ethanol extract of *H. speciosa* leaves did not inhibit the proliferation of MCF-7 (breast adenocarcinoma), LNCaP (prostate adenocarcinoma), HepG2 (hepatoma), or LU-1 (lung carcinoma) human cell lines at a final concentration of 20 μg/mL [34]. These data corroborate the results presented here. Anyway, there are no reports about the cytotoxic potential of extracts of *L. gracilis* and *H. calida* in the literature.

In summary, the active extracts were those obtained from *G. blepharophylla, G. hispida, J. curcas, K. rugosa*, and *L. gracilis.* One of the most potent cytotoxic activities was that obtained from *K. rugosa*. Additionally, seven compounds isolated from the active ex-

tracts were tested. This manuscript contributes to the understanding of the potentialities of some Brazilian plants as a source of new anticancer drugs.

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Conflict of Interest

There is no conflict to disclose.

Affiliations

[1] Department of Chemistry, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil
[2] Department of Physiology and Pharmacology, Federal University of Ceará, School of Medicine, Fortaleza, Ceará, Brazil
[3] Department of Chemistry, Federal University of Amazonas, Manaus, Amazonas, Brazil
[4] Department of Biology, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil
[5] Department of Agronomic Engineering, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil
[6] Department of Physiology, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil

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