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Screening of Latin American Plants for Cytotoxic Activity

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Abstract

The SRB cytotoxicity assay was used to screen plant extracts, in a collaborative multinational OAS project involving Argentina, Bolivia, Colombia, Costa Rica, Guatemala, Nicaragua and Panama, against breast (MCF-7), lung (H-460), and central nervous system (SF-268) human cancer cell lines. Out of 310 species tested, 23 (7.4%) plants showed cytotoxic activity at GI_{50} values $\leq 10\,\mu g/ml$. The most active plants were *Thevetia ahouai*, *Physalis viscosa*, *Piper jacquemontianum*, *Piper barbatum*, *Senna occidentalis*, *Tovomita longifolia*, and *Lippia cardiostegia*. *Blepharocalyx salicifolius* and *Senna occidentalis* were selectively active against one cell line, SF-268 or MCF-7, respectively. Within the framework of this project, 14 compounds have been isolated, 5 new (4 benzophenones, coumarin) and 9 known to the literature. But only the bioassay-guided fractionation of the active extract of *Piper barbatum* leaves, which led to the isolation of three known compounds: (2′*E*, 6′*E*)-2-farnesyl-1,4-benzoquinone (**1**), (2′*E*, 6′*E*)-2-farnesylhydroquinone (**2**), and dictyochromenol (**3**), is reported here. The chemical structures of **1** and **2** were determined by spectral means (1D, 2D NMR, MS) and chemical data. Among these three, (2′*E*, 6′*E*)-2-farnesyl-1,4-benzoquinone was the most active (MCF-7 $GI_{50} = 1.8\,\mu g/ml$; H-460 $GI_{50} = 4.8\,\mu g/ml$; SF-268 $GI_{50} = 3.5\,\mu g/ml$).

Keywords: *Blepharocalyx salicifolius*, cytotoxicity, *Latin American biodiversity*, *Piper barbatum*, *Senna occidentalis*, SRB assay, *Thevetia ahouai*.

Introduction

Cancer is a public health problem worldwide. According to WHO, 20 million people in the world suffer from cancer, a figure projected to rise to 30 million within 20 years (WHO, 2004). Cancer causes 7.1 million deaths annually (12.6% of the global total). In Latin America, cancer has been ranked as one of the first three leading causes of death (PAHO, 2002).

The plant kingdom has been a valuable source of several clinically useful anticancer agents such as vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin, and paclitaxel (Taxol). Moreover, a number of promising new anticancer agents are in clinical development at the moment, including flavopiridol [synthetic analogue of rohitukine isolated from *Dysoxylum binectariferum* Hook. F. (Meliaceae)] and combrestatin A4 phosphate [natural product isolated from *Combretum caffrum* (Eckl. & Zeyh.) Kuntze (Combretaceae)] (Cragg & Newman, 2005).

An analysis of the distribution of higher plants by continents indicates that Latin America possesses a higher number of vascular plants (85,000 plants) than tropical and subtropical Asia (50,000 plants) (World Conservation Monitoring Center, 1992). Although it was believed in the past that the forests from Southeast Asia were more diverse, there is recent evidence indicating that neotropical forests located in Latin America possess higher diversity of plants in the world (Berry, 2002).

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In order to explore rationally the potential of the plant diversity of the region, a multinational OAS project, comprising the multidisciplinary collaborative participation of research centers in Argentina, Bolivia, Colombia, Costa Rica, Guatemala, Nicaragua, and Panama, was carried out during the period 2001–2004. This project aimed at screening organic plant extracts for antifungal, anti-trypanosomal, anti-leishmanial, antimalarial, and cytotoxic activities and subsequently to isolate and characterize bioactive molecules.

For this, plants from a database of ethnomedical uses of Latin American plants, PLANMEDIA (CIFLORPAN, 2004), were selected according to the amount of biological and chemical information available in the literature for cytotoxicity screening in order to identify potential anticancer plants of the region. We report here screening of 452 plant extracts for cytotoxicity using the sulforhodamine assay and the isolation of compounds from the most active plant *Piper barbatum*. Results of antiprotozoal screening will be reported later.

Materials and Methods

Plant material

Plants were collected mainly from tropical forests in the seven countries (Argentina, Bolivia, Colombia, Costa Rica, Guatemala, Nicaragua, and Panama). Their taxonomic identity was established by the botanists Martha Gattuso, M. Frecentese, Elisa Petenatti (Argentina); Rosy de Michel, Genevieve Bourdy (Bolivia); Ricardo Callejas, Edgar Linares, José Luis Fernández, Zaleth Cordero, Santiago Díaz, N.R. Salinas (Colombia); Luis Guillermo Acosta, Luis D. Vargas, Alexánder Rodríguez (Costa Rica); Mario Veliz, Elfriede de Pöll, Juan José Castillo (Guatemala); Ricardo Rueda, Dania Paguaga, Hilario Mendoza, Nelson Toval y Miguel Garmendia (Nicaragua); Mireya Correa (Panama); and the voucher specimens are deposited at the corresponding National Herbaria in each country: Herbarium of the National University of San Luis (UNSL), Argentina, and Herbarium of the National University of Rosario (UNR), Argentina; National Herbarium of Bolivia (HLP), Bolivia; National Colombian Herbarium (COL), Colombia; Herbarium of INBio, Costa Rica; Herbarium of Farmaya, Guatemala; Universidad Nacional Autónoma de Nicaragua-León (Herbario UNAN-León; HULE), Nicaragua; and Herbarium of the University of Panama (PMA), Panama (Table 1).

Selection of plants

A random list of plants from the ethnomedical database PLANMEDIA (CIFLORPAN, 2004) was submitted to a NAPRALERT search for biological and chemical information. From that list, 314 plant species were prioritized according to the amount of information found in the literature.

Preparation of extracts

In general, plant material was macerated with 80% ethanol (24 h) for extraction (3-times). The plant extracts were filtered and concentrated *in vacuo* at $<40^{\circ}C$ in a rotary evaporator and stored at $-80^{\circ}C$ until further use.

Cytotoxicity bioassays

The cytotoxic activity against breast (MCF-7), lung (H-460), and central nervous system (SF-268) human cancer cell lines was determined according to the method of Monks et al. (1991, 1997). The cell lines were obtained from the U.S. National Cancer Institute. Adriamycin was used as standard.

Isolation of cytotoxic compounds

The leaves of *P. barbatum* (853 g) were extracted according to the procedure described above, resulting in 130 g of crude extract. The EtOH extract was then partitioned successively with $CHCl_3$, hexane, MeOH 90%, EtOAc, and BuOH. The cytotoxic activity was retained in the hexane (115 g) partition. The hexane partition was further chromatographed over silica gel with hexane: EtOAc ($0 \rightarrow 100\%$) mixtures in order of increasing polarity, followed by EtOAc:MeOH ($0 \rightarrow 100\%$) mixtures to give 126 fractions (A–V) of 500 ml each.

The active fraction L (68.1 g) was submitted to silica gel chromatography and eluated with gradients of hexane:EtOAc ($0 \rightarrow 100\%$) and EtOAc:MeOH ($0 \rightarrow 100\%$) to give 39 fractions (L-1 to L-10) of 250 ml each.

The major component of fraction L-4 (419.5 mg) was subjected to a silica gel column chromatography with solvent gradients of toluene-EtOAc ($0 \rightarrow 100\%$), followed by further purification using Sephadex column in $CHCl_3$ 100% to give compound **1** (52 mg).

The active fraction L-5 (2.2 g) was further chromatographed on silica gel 60 (0.063–0.200 mm) with a solvent gradient of hexane-CH_2Cl_2 ($0 \rightarrow 100\%$). Further purification of two main components by Sephadex LH-20 in $CHCl_3$ 100% was carried out to afford two main fractions of 10 ml each (L-5a and L-5b). Crystallization of fraction L-5a with $CHCl_3$ 100% afforded compound **2** (120 mg). Fraction L-5b furnished compound **3** (214.7 mg).

Results and Discussion

Ethnomedical database

This collaborative multinational OAS project aimed at discovering novel bioactive molecules during 2001–2004

Table 1. Cytotoxic extracts of selected plants.

Plant: FAMILY/species	Vernacular name	Voucher no./date and place of collection	Part	Solvent	GI_{50} (μg/ml)		
					MCF-7	H-460	SF-268
ANNONACEAE							
Annona muricata L.	Guanábana (Colombia, Panamá)	COL 504946; 07/31/02, Vereda Andalucía, Anapoima–Cundinamarca; Colombia	LF	EtOH 95%	6.2	4	8.5
Unonopsis theobromifolia N. Zamora & Poveda		LA 3475; 10/06/04, La Cangreja National Park; Costa Rica	LF	MeOH 98%	7.7	4.9	>10
APOCYNACEAE							
Thevetia ahouai (L.) A. DC.	Huevita de gato, habano, cobalonga (Colombia), and huevos de gato (Guatemala)	8645; 9/98, Cerro Cahui, Peten; Guatemala	ST	EtOH 95%	0.47	0.29	0.52
ASTERACEAE							
Chromolaena leivensis (Hieron.) R.M. King & H. Rob.	Curalotodo	COL 504952; 07/31/02, 50 m ahead of peaje Mondoñedo, La Mesa–Cundinamarca highway; Colombia	AP	EtOH 95%	7.5	7.1	7.5
Melampodium divaricatum (Rich.) DC.	Tres puntas, dunguina (Guatemala)	FLORPAN 6283; 09/01/03, Soberania National Park, Oleoducto trail, 4.8 km of distance; Panama	ST	EtOH 80%	8.5	8.4	>10
BORAGINACEAE							
Cordia cylindrostachya (Ruiz & Pav.) Roem. & Schult.	Leño negro, hierba de golondrina	COL 498048; 03/03/04, Vía Bogotá-Mesitas del Colegio–Cundinamarca; Colombia	LF	EtOH 95%	5.9	5.7	8.2
CLUSIACEAE							
Hypericum uliginosum Kunth	Mil flores	970; 11/02, San Marcos; Guatemala	LF	EtOH 95%	9.8	>10	6.4
Tovomita longifolia (Rich.) Hochr.		FLORPAN 5328; 09/13/01 Cerro Jefe; Panama	LF	$CHCl_3$	3.2	5.5	7.2
FABACEAE							
Enterolobium contortisiliquum (Vell.) Morong	Timboi, toco, timboy	RM2560; 05/01, La Brecha–Aguarati zone, Department of Santa Cruz; Bolivia	SB	EtOH	>10	4.6	5.6
FABACEAE-CAESALPINIOIDEAE							
Senna occidentalis (L.) Link	Furrusca (Colombia), totekumanda (Bolivia), frijolilo (Panama)	RM2670; 05/01, zone between Brecha and Aguarati; Bolivia	LF	EtOH	4.2	5.4	3.9
MELASTOMATACEAE							
Monochaetum myrtoideum (Bonpl.) Naudin	Saltón	COL 498053; 05/03/04, La Mesa–Mosquera highway, Cundinamarca; Colombia	LF	CH_2Cl_2	10	>10	>10
MYRICACEAE							
Myrica pubescens Humb. & Bonpl. ex Willd.	Cerillo	LA 3454; 06/24/04. Irazú Volcano National Park, Prusia; Costa Rica	LF	MeOH 98%	9	8.7	>10

MYRTACEAE							
Blepharocalyx salicifolius (Kunth) O. Berg.	Anacahuita	MG 427 UNR; 03/15/02, Rosario; Argentina	LF	MeOH	9	>10	>10
PIPERACEAE							
Piper jacquemontianum Kunth	Cordoncillo	959; 6/2002, Samayac; Guatemala	HB	EtOH	3.9	4.9	4.6
Piper barbatum Kunth	Cordoncillo	COL 499616; 03/11/03, La Mesa–Mosquera highway, Cundinamarca; Colombia	AP	EtOH	3.3	3.7	3.9
			LF	EtOH	1.4	1.5	1.5
			FR	EtOH	1.75	2.05	1.95
Piper glabratum Kunth	Thuda	GB 1877; 07/02, Santa Rosa de Maravilla zone; Bolivia	LF	CH_2Cl_2	8	5.9	6.4
QUIINACEAE							
Quiina florida Tul.	Coloradillo (Cochabamba, Bolivia), Masarandava de altura (Beni)	AR 16820 H; 06/03, Santa Rosa de Maravilla zone; Bolivia	BK	H_2O	>10	10	>10
RUBIACEAE							
Coutarea hexandra (Jacq.) K. Schum	Mariangola (Colombia), quina de piauhy, cascarilla, quina do río (Argentina), palo amargo, quinita (Guatemala), müin kirire	FLORPAN 5485; 01/31/02, Podocarpus trail, forest keepers, Altos de Campana National park; Panama	FR	EtOH 80%	10	>10	>10
SOLANACEAE							
Physalis viscosa L.	Huevo de gallo	UNSL 8579; 03/01/03, Merlo, Departament of Junín, San Luis; Argentina	AP	CH_2Cl_2	3.2	3.3	7
THEOPHRASTACEAE							
Bonellia nervosa (C. Presl) B. Ståhl & Källersjö	Siempre viva, burriquita	LA 0202; 8/12/99 Bagaces of Guanacaste, Lomas de Barbudal Biological Reserve; Costa Rica	AP	MeOH 98%	9.2	5.4	9.7
TILIACEAE							
Trichospermum galeottii (Turcz.) Kosterm.		FLORPAN 2304; 11/23/95, Altos de Campana, National Park, 500 m before the entrance; Panama	BK	$CHCl_3$	4.3	4.9	>10
VERBENACEAE							
Lippia cardiostegia Benth.		AR 6763; 11/16/00, Garabito of Puntarenas; Costa Rica	RT	MeOH 98%	5.5	5.2	7.5
ZYGOPHYLLACEAE							
Larrea cuneifolia Cav.	Jarilla macho	UNSL 6437; 03/01/04, Sierras de las Quijadas, San Luis; Argentina	AP	MeOH	8.2	9.9	>10
Adryamicin					6.2×10^{-7}	3.6×10^{-7}	5.3×10^{-7}

AP, aerial parts; BK, bark; FR, fruit; LF, leaf; RT, root; ST, stems; SB, bark of stem; HB, herb. GI_{50}, growth inhibition of 50% of cells.

Table 2. Inactive plant extracts.

Plant: FAMILY/species	Vernacular name	Voucher no./date and place of collection	Part	Solvent	GI_{50} (μg/ml) MCF-7	H-460	SF-268
AGAVACEAE							
Agave angustifolia Haw.	Maguey	LA 0940; 04/26/00; Santa Rosa National Park; Costa Rica	AP	MeOH 98%	42	37	43
ANNONACEAE							
Xylopia aromatica (Lam.) Mart.	Malagueto	COL 468667; 09/25/01; Cunday–Cundinamarca; Colombia	LF	EtOH 95%	46	50	44
ARALIACEAE							
Dendropanax arboreus (L.) Decne. & Planch.		Rueda 17354; 09/09/2002; Miraflor Natural Reserve, Community Puertas Azules, Estelí; Nicaragua	LF	EtOH 95%	36	33	41
ASTERACEAE							
Baccharis medullosa DC.	Carqueja	4931 (UNSL); 12/03; San Luis, Dpto. La Capital, El Chorrillo. 820 ms · m.; Argentina	AP	MeOH	42	45	46
Baccharis notosergila Griseb.	Tipisha	7796 (UNSL); 11/03; Buenos Aires, Pdo. De Luján, close to ciudad homónima; Argentina	AP	MeOH	11	12	24
Calea jamaicensis (L.) L.	Carrasposa	COL 468655; 07/27/01; Cunday/Cundinamarca; Colombia	AP	EtOH 95%	45	40	51
Critonia morifolia (Mill.) R.M. King & H. Rob.	Lengua de vaca	COL 504880; 03/11/03; km. 73, Carretera Mosquera—La Mesa, Cundinamarca; Colombia	FR	EtOH 95%	18.5	19	18.5
Gochnatia glutinosa (Don) D. Don ex Hook. & Arn.	Jarilla	7461(UNSL); 12/02; San Luis, Dpto. Belgrano, La Calera; Argentina	LF; ST	EtOH 95%	13	16	22
Mikania periplocifolia Hook. & Arn.	Enriedo	8521(UNSL); 12/02; San Luis, Dpto. Pringles, Valle de Pancanta; Argentina	AP	EtOH 95%	15	15	13
Neurolaena lobata (L.) R. Br. ex Cass.	Gavilana, contragavilana, jua kaibit, ina kinkub-gid	FLORPAN 6276; 08/27/03; Cerro Jefe Section, Chagres National Park; Panama	LF	EtOH 80%	46	48	84
Pterocaulon polystachyum DC.	Sombra de toro0	1497 UNR; 09/04; Chaco santafesino, Argentina	AP	CH_2Cl_2	19	25	26
Solidago chilensis Meyen	Vara de oro	MG 257 UNR; 11/03; Perez, Argentina	LF	MeOH	15	16	39
			FW	MeOH	13	15	11
Tithonia diversifolia (Hemsl.) A. Gray	Quil, Girasol de monte	Farmaya 684; 01/8/02; Samayac; Guatemala	LF	EtOH 95%	35	33	53
Vernonanthura deppeana (Less.) H. Rob.	Suquinay	Farmaya 878; 01/4/01; Samayac; Guatemala	LF	EtOH 95%	38	36	64
CELASTRACEAE							
Crossopetalum tonduzii (Loes.) Lundell		LA 3309; 01/14/04; Braulio Carrillo National Park; Costa Rica	RT	MeOH 98%	15	21	28
CLUSIACEAE							
Chrysochlamys eclipes L.O. Williams		FLORPAN 6431; 12/17/03; plot in Altos de Campana National park; Panama	ST	EtOH 80%	12	11	14
			LF	EtOH 80%	16	16	19
CONVOLVULACEAE							
Ipomoea cairica (L.) Sweet	Campanilla	6259(UNSL); 12/03; Dpto. La Capital, ciudad; San Luis; Argentina	WP	MeOH	18	21	12

CUCURBITACEAE							
Psiguria warscewiczii (Hook. f.) Wunderlin	Ni murei odochi	FLORPAN 5895; 3/13/03; Dichapetalaceae trail, Altos de Campana National Park; Panama.	WP	EtOH 95%	26	33	30
EBENACEAE							
Diospyros digyna Jacq.		LA 1124; 02/05/00; Guanacaste National Park; Costa Rica.	RT	MeOH 98%	14	13	30
FABACEAE							
Caesalpinia paraguariensis (D. Parodi) Burkart	Ivirayepiro	RM2571; 05/01; Ibasiriri, Santa Cruz; Bolivia	BK	EtOH 95%	14	11	15
Dipterix oleifera Benth.		Rueda 17563; 12/19/02; Refugio Bartola, San Juan River, Nicaragua	LF	EtOH 95%	27	20	22
Prosopis nigra (Griseb.) Hieron.	Algarrobo negro	1449 UNR; 12/02; Rosario, Santa Fe, Argentina	AP	MeOH	20	25	35
HYDROPHYLLACEAE							
Wigandia urens var caracasana (Kunth) D.N. Gibson	Chocón	Farmaya 347; 06/05/04; Mixco; Guatemala	FW	EtOH 95%	17	16	19
MALPIGHIACEAE							
Banisteriopsis muricata (Cav.) Cuatrec.		FLORPAN 5519 ; 07/25/02; Shell gasoline station in Capira, Panama	LF	EtOH 80%	56	53	75
MELASTOMATACEAE							
Miconia buxifolia Naudin	Gallinazo	COL 471732; 06/11/01; Vía Mosquera–La Mesa/Cundinamarca; Colombia	LF	EtOH 95%	42	43	44
MYRTACEAE							
Blepharocalyx gigantea Lillo	Horco molle	MG 815 UNR; 11/03; Tucuman; Argentina	LF	MeOH	30	68	32
PAPAVERACEAE							
Bocconia frutescens L.	Trompeto	COL 510530; 03/11/03; Mosquera highway–La Mesa; Colombia	LF	EtOH 95%	48	49	46
PHYTOLACCACEAE							
Petiveria alliacea L.	Anamú	HPUJ 011749; 06/05/01; Viotá–Cundinamarca; Colombia	LF	EtOH 95%	22	22	42
Phytolacca bogotensis Kunth	Ombusillo	MG 121 UNR, 11/02; Gral. Belgrano. Misiones; Argentina	LF	CH_2Cl_2	34	35	30
PIPERACEAE							
Piper acutifolium Ruiz & Pav.	Thuda	GB1640; 06/02; Santa Rosa de Maravilla, La Paz; Bolivia	LF	CH_2Cl_2	36	25	27
Piper aduncum L.	Thuda	GB1578; 06/02; Santa Rosa de Maravilla, La Paz; Bolivia	LF	CH_2Cl_2	27	25	23
Piper elongatum Vahl	Matico	GB1644; 06/02; Santa Rosa de Maravilla, La Paz; Bolivia	LF	CH_2Cl_2	15	13	13
Piper heterophyllum Ruiz & Pav.	Thuda	NF1200; 06/02; Santa Rosa de Maravilla, La Paz; Bolivia	LF	CH_2Cl_2	29	26	22
Piper hispidum Sw.	Thuda	NF1300; 06/02; Santa Rosa de Maravilla, La Paz; Bolivia	LF	CH_2Cl_2	17	14	16
Piper holtonii C. DC.	Cordoncillo	COL 468663; 06/01/01; El Triunfo/Cundinamarca; Colombia	RT	EtOH 95%	12	11	13

(*Continued*)

Table 2. Continued.

Plant: FAMILY/species	Vernacular name	Voucher no./date and place of collection	Part	Solvent	GI_{50} (μg/ml) MCF-7	H-460	SF-268
Piper longestylosum C. DC.	Thuda	NF1500; 06/02; Santa Rosa de Maravilla, La Paz; Bolivia	LF	CH_2Cl_2	27	24	24
Piper pilirameum C. DC.	Thuda	GB1716; 06/02; Santa Rosa de Maravilla, La Paz; Bolivia	LF	CH_2Cl_2	17	14	15
Piper rusbyi C. DC.	Thuda	GB1614; 06/02; Santa Rosa de Maravilla, La Paz; Bolivia	LF	CH_2Cl_2	18	13	16
RANUNCULACEAE							
Clematis montevidensis Spreng.	Cabello de angel	5061(UNSL); 12/03; Dpto. La Capital, Rodeo del Alto; San Luis; Argentina	AP	CH_2Cl_2	38	56	34
RHAMNACEAE							
Ziziphus mistol Griseb.	Yuat	GB1977; 05/01; Aguarati, Santa Cruz; Bolivia	LF	EtOH 95%	17	15	14
RUBIACEAE							
Chiococca alba (L.) Hitchc.		FLORPAN 6200; 07/22/03; Punta Chame; Panama	LF	EtOH 80%	78	>100	>100
Palicourea guianensis Aubl.	Suidir mas	FLORPAN 6549; 05/06/04; beside the highway in Soberania National Park; Panama	ST	EtOH 80%	20	22	47
Psychotria psychotriifolia (Seem.) Standl.		FLORPAN 5923; 04/11/2003; Guanche River, Colón; Panama	LF	EtOH 80%	24	53	94
			ST	EtOH 80%	19	46	>100
Richardia scabra L.		FLORPAN 6272; 08/27/03; Cerro Jefe section, Chagres National Park, Panama	RT	EtOH 80%	>100	>100	>100
SMILACACEAE							
Smilax domingensis Willd.	Zarzaparrilla	Farmaya 662; 01/09/01; Samayac; Guatemala	RZ	EtOH 95%	16	12	27
SOLANACEAE							
Cestrum parqui L'Hér.	Duraznillo negro	8780 (UNSL); 11/02; San Luis, Dpto. La Capital, The Vulcano, Argentina	AP	CH_2Cl_2	32	22	32
Cestrum nocturnum L.	Galan de noche	FLORPAN 5922; 11/4/03; Guanche river, Colon; Panama	LF	EtOH 95%	21	15	20
Cestrum megalophyllum Dunal	Tinto	COL 507235; 07/27/04; Mosquera–La Mesa Highway; Colombia	FR	EtOH 95%	33	29	38
Physalis viscosa L.	Camambú	8579 (UNSL); 12/02; San Luis, Dpto. Junín, Merlo, close to the town, Argentina	Fresh AP	MeOH	16	23	45
			Dried AP	MeOH	14	12	26
STERCULIACEAE							
Sterculia apetala (Jacq.) H. Karst.	Castaño, Mano de león	Farmaya 280; 01/11/02; Peten; Guatemala	BK	EtOH 95%	23	25	30
VALERIANACEAE							
Valeriana prionophylla Standl.	Valeriana	Farmaya 590; 01/10/01; Quiché; Guatemala	RT	EtOH 95%	35	38	91
		Farmaya 701; 01/10/02; Quiché; Guatemala	LF	EtOH 95%	38	31	41

AP, aerial parts; BK, bark of stem; FR, fruit; LF, leaf; RZ, rhizomes; RT, roots; ST, stems; FW, flowers; WP, whole plant. GI_{50}, growth inhibition of 50% of cells.

has firstly resulted in the generation of an ethnomedical database PLANMEDIA with a total of 4129 entries corresponding to 1152 species (167 families and 692 genera). The establishment of this database was intended to keep vast knowledge in traditional medicine in Latin America mentioned by Pedersen and Baruffati (1985). This is supported by the fact that Latin America has a high percentage of native indigenous people who depend upon traditional medicine for treating their ailments (Gupta, 2005).

Cytotoxicity of extracts

In addition, from 311 plant species collected, 452 extracts were prepared and tested in the cytotoxicity assay. Out of 452, 81 extracts were tested in five concentrations (100, 31.6, 10.0, 3.17, 1.0 μg/ml) to determine the GI_{50} values (Tables 1 and 2). Forty-nine percent of plants tested at this stage belong to the major families such as Asteraceace (18%), Piperaceae (16%), Rubiaceae (5%), Solanaceae (5%), and Fabaceae (5%). The extracts with GI_{50} values $\leq$10 μg/ml were considered as active. Twenty-five (5.5%) extracts (Fig. 1; Table 1), representing 23 species, 21 genera, 18 families, showed relevant cytotoxic activity. The cytotoxic activities of extracts distributed in three ranges of concentration (5–10 μg/ml, 1–5 μg/ml, $\leq$1 μg/ml) are shown in Figure 2. The more active cytotoxic extracts in the biological screening are summarized in Table 1.

The most active plant against the three cancer cell lines was *Thevetia ahouai* with the following GI_{50} values [MCF-7 (μg/ml): 0.47; H-460 (μg/ml): 0.29; SF-268 (μg/ml): 0.52]. Two plants showed selectivity against one specific cancer cell line: *Blepharocalyx salicifolius* IC_{50} (μg/ml) 9.0 MCF-7 and *Senna occidentalis* IC_{50} (μg/ml) 3.9 SF-268.

Thevetia ahouai and *Annona muricata* reported ethnomedical use against cancer in several Latin American countries (Gupta et al., 1986; García Barriga, 1992). The ethnomedical use of these two plants may be corroborated with their cytotoxic activity found in our biological screening (Table 1). This is further supported

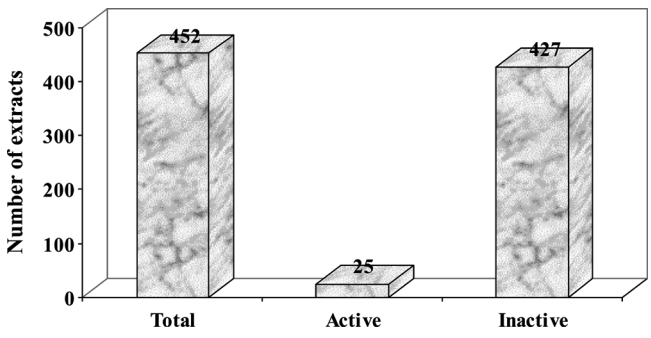

Figure 1. Total number of extracts tested versus active and inactive extracts.

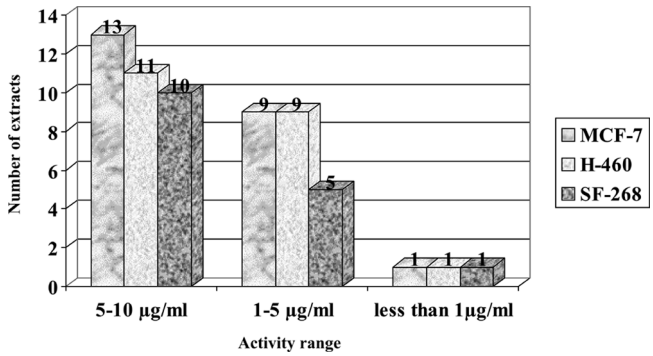

Figure 2. Cytotoxic activity of extracts in three ranges of concentration.

by the presence of known cytotoxic compounds in these plants, as follows: (a) three cardenolide glycosides, neriifolin, 3′-*O*-methylevomonoside and 2′-acetyl-neriifolin, have been isolated from *T. ahouai*. These compounds exhibited a distinctive pattern of differential cytotoxicity in the National Cancer Institute's human disease–oriented 60-cell-line tumor screening panel (Decosterd et al., 1994); (b) *A. muricata*, like all plants of the family Annonaceae, contains acetogenins. There are several reports on cytotoxic mono and bis-tetrahydrofuran acetogenins isolated from *A. muricata* (Rieser et al., 1996; Kim et al., 1998; Chang & Wu, 2001; Liaw et al., 2002; Chang et al., 2003; Suksamrarn et al., 2004).

Flavanones and flavones found in the genus *Chromolaena* showed moderate cytotoxicity against human small-cell lung cancer (NCl-H187) cells (Suksamrarn et al., 2004). This evidence in the literature and cytotoxic activity [IC_{50} values MCF-7 (μg/ml): 7.5; H-460 (μg/ml): 7.1; SF-268 (μg/ml): 7.5] of *Chromolaena leivensis* in the current study may corroborate the ethnomedical use of species of genus for the treatment of tumors (Gupta, 1995).

Cytotoxic activities of *Enterolobium contortisiliquum* against BAC1.2F5 mouse macrophages, EL-4 mouse lymphoma cells, and L-929 mouse fibroblasts have been reported due to the presence of bisdemosidic saponin (Mimaki et al., 2003, 2004). This type of saponin could be responsible for the cytotoxicity shown against H-460 and SF-268 cell lines in the current study.

Physalins (13,14-*seco*-16,24-cyclosteroids) from *Physalis* species have shown cytotoxic activity against HeLa cells (Makino et al., 1994, 2003; Kawai et al., 2002). A cytotoxic flavonoid glycoside isolated from *Physalis angulata* L. showed remarkable cytotoxicity *in vitro* against murine leukemia cell line P-388, epidermoid carcinoma of the nasopharynx KB-16 cells, and lung adenocarcinoma A-549 (Ismail & Alam, 2001). *Physalis viscosa*, as member of the cytotoxic *Physalis* genus, displayed cytotoxicity against the three cell lines in the current work.

In the genus *Piper*, pyridone alkaloids exhibited cytotoxicity against P-388, HT-29, or A549 and KB cell lines *in vitro* (Duh et al., 1990; Chen et al., 2003), and amides exerted cytotoxicity (cell survival $<15\%$) against CCRF-CEM, HL-60, PC-3, and HA22T cell lines (Chen et al., 2002). As part of this genus *Piper*, *P. jacquemontianum*, *P. barbatum*, and *P. glabratum* showed significant cytotoxicity against the three cell lines.

Two new [(*E*)-3-(2-hydroxy-7-methyl-3-methyleneoct-6-enyl)-2,4,6-trihydroxybenzo phenone; 8-benzoyl-2-(4-methylpenten-3-yl)chromane-3,5,7-triol] and one known (4-geranyloxy-2,6,-dihydroxybenzophenone) benzophenones isolated from *Tovomita longifolia* presented cytotoxicity against the three cell lines in this project (Pecchio et al., 2006). Previously, two known xanthones, trapezifolixanthone and manglexanthone, were isolated as cytotoxic constituents from the $CHCl_3$ extract of the roots of other *Tovomita* species by bioassay-guided fractionation using the KB cell line (Seo et al., 1999).

As part of this project, three cytotoxic cucurbitacins (23,24-dihydro cucurbitacin F, 23,24-dihydro-25-acetylcucurbitacin F and 2-O-β-D-glucopyranosyl-23,24-dihydrocucurbitacin F) were found in fruits of *Courtarea hexandra* (Olmedo et al., 2005).

Currently, no data are available regarding the *in vitro* cytotoxic activity for the species or genera of *Blepharocalyx salicifolius*, *Cordia cylindrostachya*, *Coutarea hexandra*, *Hypericum uliginosum*, *Jacquinia nervosa*, *Larrea cuneifolia*, *Lippia cardiostegia*, *Melampodium divaricatum*, *Monochaetum myrtoideum*, *Myrica pubescens*, *Senna occidentalis*, *Trichospermum galeottii*, and *Unonopsis theobromifolia*, and thus are interesting for further studies.

Identification of isolated compounds

Compound **1** was identified as (2′*E*, 6′*E*)-2-farnesyl-1,4-benzoquinone, **2** as (2′*E*, 6′*E*)-2-farnesylhydroquinone, and **3** as dictyochromenol by comparison of their spectral data (Table 3) with those reported in the literature (Muanza-Nkongolo et al., 1984; Peña et al., 2000). All three compounds are known compounds. Compounds **1** and **2** have been previously found in fruits of *Piper barbatum* C. DC. (synomym: *Piper bogotense* C. DC.) (Peña et al., 2000) but not in the leaves, which was the part studied in this project. Compound **2** was isolated for the first time from the brown seaweed *Dictyopteris undulata* Holmes (Ochi et al., 1979) and subsequently from *Wigandia kunthii* Choisy (Hydrophyllaceae) (Gomez, 1980). Compound **3** has been only reported in the brown alga *Dictyopteris undulata* (Muanza-Nkongolo et al., 1984). This is the first report of its presence in higher plants.

Within the framework of this study, 14 compounds have been isolated, 5 new (4 benzophenones, 1 coumarin) and 9 known to the literature.

Table 3. ^{1}H and ^{13}C NMR spectroscopic data for compounds **1, 2, 3**.

	1		**2**		**3**	
Position	δC	δH	δC	δH	δC	δH
1	187.9		148.8		149.7	
2	148.5		138.5		122.0	
3	132.3	6.50 q, J = 1.8, 2.1	113.8	6.5 m	113.1	6.5 m
4	187.6		149.3		149.7	
5	136.7	6.75 m	116.7	6.5 m	115.6	6.7 m
6	136.3	6.72 m	121.4	5.15 m	116.4	6.7 m
7	27.4	3.15 d, J = 7.1	27.4	3.1 m	123.9	5.1 m
8	117.7	5.10 m	117.6	6.6 m	131.2	5.6 d, J = 1.5
9	140.1		135.5		146.7	
10	26.4	1.95 m	39.7	1.9 m	41.0	2.0 m
11	26.7	1.95 m	29.6	3.3 m	22.6	2.2 m
12	124.3	5.10 m	123.8	5.3 m	122.7	6.2 d, J = 1.5
13	135.4		132.4		135.2	
14	39.7	1.95 m	39.7	1.9 m	39.6	1.7 m
15	26.7	1.95 m	26.7	2.04 m	26.9	2.1 m
16	124.3	5.10 m	124.4	5.1 m	122.2	6.2 d, J = 1.5
17	131.3		128.4		135.2	
18	25.9	1.67 s	26.4	1.67 s	25.9	1.4 s
19	17.7	1.59 s	17.7	1.58 s	17.6	1.65 s
20	16.0	1.59 s	16.0	1.57 s	15.9	1.65 s
21	16.1	1.63 s	16.0	1.73 s	25.7	1.7 s

300 MHz for ^{1}H-NMR, 75 MHz for ^{13}C; solvent: $CDCl_3$ and chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as internal standard and coupling constants are given in Hz.

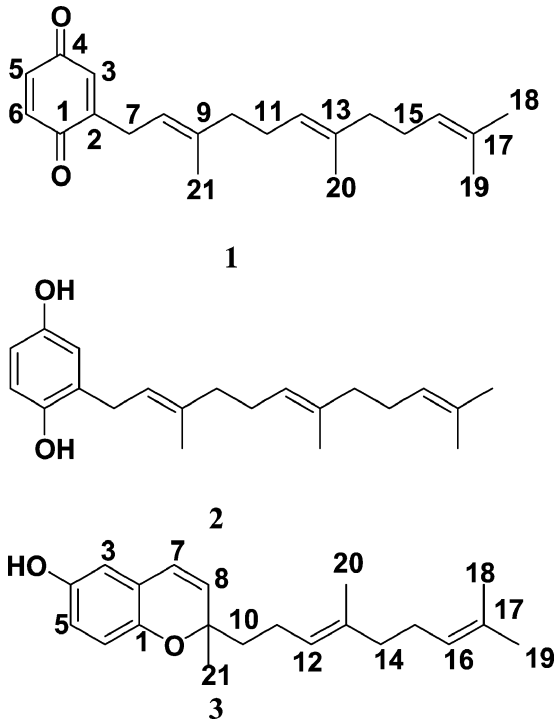

Figure 3. Compounds isolated from *P. barbatum*.

Cytotoxicity of isolated compounds

Compound **1** showed the highest cytotoxic activity (GI_{50} 1.8 μg/ml) against the MCF-7 cancer cell line. There is no significant difference for the cytotoxic activity against H-460 among the three compounds (Table 2). Furthermore, compound **2** displayed more activity (GI_{50} 1.6 μg/ml) against SF-268 than compounds **1** and **3**, which showed similar activities against this cancer cell line. The isolated compounds are shown in Figure 3.

Although compounds **1** and **2** were more active against one cell line (MCF-7 or SF-268, respectively) than the others, there is not enough evidence of selectivity from these data. Further investigation of their cytotoxic activity against other cancer cell lines needs to be carried out.

This is the first report of the *in vitro* cytotoxic activities against the human cancer cell lines MCF-7, H-460 and SF-268 of (2′*E*,6′*E*)-2-farnesyl-1,4-benzoquinone (**1**), (2′*E*,6′*E*)-2-farnesylhydroquinone (**2**), and dictyochromenol (**3**).

Conclusions

This study has identified a number of plant extracts (Table 1) that have shown *in vitro* cytotoxic activities, based on the selection of plants present in the database PLANMEDIA. The results of the cytotoxicity screening revealed that 3 out of the 23 active plants belong to the genus *Piper*. In the *Thevetia ahouai* and *Annona muricata* species examined, their ethnomedical uses were confirmed with the cytotoxic activity presented in the screening.

Acknowledgments

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