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Evaluation of the activity of Guatemalan medicinal plants against cancer cell lines and microbes

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Medicinal plants are used by rural Guatemalan villagers to treat a variety of ailments, and a better understanding of their effectiveness against common diseases is warranted. Acetone and methanol extracts of 73 medicinal plant species from 44 families were bio-assayed against breast, cervical, skin, and tongue cancers, and *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*. Half-maximum inhibitory concentrations (IC_{50}) and half-maximum cytotoxicity concentrations (CC_{50}) were determined against cancerous and non-cancerous cell lines, respectively. Minimum inhibitory concentrations (MIC) were determined for active extracts. *Bursera simaruba* (L.) Sarg. (Burseraceae), *Byrsonima crassifolia* (L.) Kunth (Malpighiaceae), *Guazuma ulmifolia* Lam. (Malvaceae), and *Quercus acatenangensis* Trel. (Fagaceae) were inhibitory to one or more cancer cell lines and yielded promising IC_{50} and CC_{50} values. *Eucalyptus globulus* Labill. (Myrtaceae), *Liquidambar styraciflua* L. (Altingiaceae), *Pelargonium hortorum* L.H. Bailey (Geraniaceae), and *Psidium guajava* L. (Myrtaceae) were inhibitory to one or more microbes and had MIC's of 250 µg/ml or less against one or more microbes. The activity of these species against cancer and pathogenic microbes indicates that they are valuable resources that should be conserved and considered for future research.

Key words: Anticancer, antimicrobial, IC_{50}, CC_{50}, minimum inhibitory concentrations (MIC), Guatemala.

INTRODUCTION

Economic constraints and limitations in the accessibility and availability of Western biomedical knowledge reduce health care options for rural Guatemalans to primarily traditional approaches (Goldman et al., 2002; Adams and Hawkins, 2007; Hautecoeur et al., 2007). While the loss of traditional knowledge among indigenous peoples has been documented in Guatemala (Comerford, 1996; Kufer et al., 2005), and worldwide (Chaudhuri, 2007; Newman

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et al., 2008), there remains a significant level of medicinal plant use among rural Guatemalans (Booth et al., 1993; Comerford, 1996; Kufer et al., 2005; Cáceres, 2009). However, relatively few have been screened for activity against a range of microbes and cancer cell lines. Kufer et al. (2005) and Adams and Hawkins (2007) noted that there is a need for scientific investigations of Guatemalan medicinal plants in order to determine their activity against human diseases. These observations along with concerns regarding the evolution of drug resistant micro-organisms and cancer cell lines (Chivian and Bernstein, 2008; Kingston, 2011; Lambert et al., 2011; Lai et al., 2012) provided the basis for this study.

Acetone and methanol extracts of 73 medicinal plant species commonly used by villagers in Chiquimula, Guatemala were chosen for microwell dilution bioassays against four cancerous cell lines, four pathogenic bacteria, and one infectious yeast. For extracts with growth inhibition values of 60% or greater, half-maximum inhibitory concentrations (IC_{50}) for cancerous cell lines, half-maximum cytotoxicity concentrations (CC_{50}) for a non-cancerous cell line, and minimum inhibitory concentrations (MIC) for microbial species were determined. Results from this study support the traditional uses of some species [for example, *Eucalyptus globulus* Labill. (Myrtaceae) and *Psidium guajava* L. (Myrtaceae)] (Comerford, 1996; Kufer et al., 2005) and revealed that several species have activity against cancerous cell lines. In addition, this study supports the belief that traditional medicinal plant species in Guatemala are valuable resources that merit conservation (Kingston, 2011).

MATERIALS AND METHODS

Study sites and plant collection

Plant species were selected based on information gathered from surveys of rural villagers from the communities of Tuticopote Abajo, El Roblarcito, San Francisco Chancó, Salitrón, and Corral de Piedra. Details regarding collection of samples as to sites, topography, vegetation associations, soil types, climate, and village cooperation as found in Galvez (2008) and Ardon (2008). For the 73 species analyzed, only the tissue used medicinally was collected (Table 1). Samples were shipped on dry ice to Brigham Young University (BYU) and stored at -80°C. Vouchers for plant identification are located in the Herbaria at Centro Universitario de Oriente, Universidad de San Carlos de Guatemala, Chiquimula, Guatemala (CUNORI) and at Brigham Young University (BRY), Provo, UT.

Tissue extraction and drug preparation

Five grams of plant tissue were ground in liquid nitrogen and then extracted sequentially with hexane, acetone and methanol. Because essential oils from these plants were extracted and assayed independent of this study, hexane extracts containing oils, fats, and waxes were discarded. Acetone and methanol extracts were filtered through cheesecloth and dried using nitrogen gas to reduce oxidation. Dried extracts were dissolved in double-distilled water (ddH_2O) to a final concentration of 8 mg/ml.

Cell lines

The human cancer cell lines used were breast (ATCC HTB-22, breast mammary gland adenocarcinoma; ATCC, Manassas, VA), HeLa (ATCC CCL-2, cervix epithelial adenocarcinoma; ATCC), skin (ATCC CRL-1619, epithelial malignant melanoma; ATCC), and tongue (ATCC CRL-2095, human epithelial squamous carcinoma; ATCC). Cytotoxicity was determined using a non-cancerous Vero cell line (ATCC CRL-1586, epithelial kidney monkey; ATCC). Cells were cultured in Dulbecco's modified eagle medium (DMEM; GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, ATCC), 1% 1 M 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) (Hyclone, Logan, UT), 1% 100 mM sodium pyruvate (Sigma-Aldrich, St. Louis, MO), and 0.5% of 10 mg/ml gentamycin (Sigma-Aldrich).

Sulforhodamine B assay

To determine the level of inhibition of plant extracts against cancer cell lines, 96-well plates were prepared for bioassay by seeding wells with the appropriate number of cells for each cell line in a total volume of 150 µl (4.0×10^4 cells/well for breast, 2.0×10^4 cells/well for HeLa, 5.0×10^4 cells/well for skin, 5.0×10^4 cells/well for tongue, and 1.5×10^4 cells/well for Vero). After incubation at 37°C for 24 h, the cells were treated with 200, 100, and 50 µg/ml of extract in triplicate and further incubated for 24 h at 37°C. Inhibition of cell growth was determined using the sulforhodamine B assay following Skehan et al. (1990) and Donaldson et al. (2004). Results in Table 2 are reported only for the 200 µg/ml concentration.

Neutral red (NR) assay

Extracts that showed inhibition levels greater than 60% at 200 µg/ml in the sulforhodamine B assay were tested using a neutral red (NR) assay (Putnam et al., 2002). Cells were seeded in 96-well plates in the same density as noted in the sulforhodamine B assay and treated with serial dilutions (200, 100, 50, 25, 12.5 and 6.25 µg/ml) of plant extract in triplicate. Additional concentrations of extract were included in the NR assay so that more data would be available for accurate calculation of half-maximum inhibitory concentrations (IC_{50}) and half-maximum cytotoxicity concentrations (CC_{50}). The IC_{50} and CC_{50} values were obtained using dosage response curves.

Microbial cultures

Staphylococcus aureus (ATCC 6538P; Becton Dickinson Laboratories, Cockeysville, MD), *Escherichia coli* (ATCC 11229; ATCC), oral isolates of *Streptococcus mutans* (ATCC 33402, ATCC), *Lactobacillus acidophilus* (ATCC 11975, ATCC), and *Candida albicans* (ATCC 90028, ATCC) were used to determine the antimicrobial activity of acetone and methanol extracts. *S. aureus*, *E. coli*, and *S. mutans* were cultured in tryptic soy broth (TSB; Becton, Dickinson and Co., Sparks, MD), *L. acidophilus* in MRS broth (Becton, Dickinson and Co.), and *C. albicans* in Sabouraud dextrose broth (SDB; Sigma-Aldrich).

Microbial inhibition bioassay

To determine which extracts exhibited inhibition against the pathogens, a microwell dilution bioassay was performed using 1000, 500, and 250 µg/ml of extract following Shrestha and St. Clair (2013). Each extract was tested in triplicate and values noted in Table 4 are percent inhibition at the 1000 µg/ml concentration.

Table 1. Scientific names, common names, voucher numbers, and use of medicinal plants.

Genus/species	Family	Common name	Voucher number#	Medicinal use (Tissue extracted)†
Acalypha guatemalensis Pax & K. Hoffm.	Euphorbiaceae	Hierba del cáncer	01-0012	Wounds, prevent scarring (L)
Anacardium occidentale L.	Anacardiaceae	Marañon	03-0010	Dysentery (L)
Baccharis latifolia (Ruiz & Pav.) Pers.	Asteraceae	Conrrodo negro	02-0015	Nerves, anxiety (AP)
Bixa orellana L.	Bixaceae	Achiote	03-0001	Diarrhea (PS)
Bougainvillea glabra Choisy	Nyctaginaceae	Buganvilea	05-0001	Respiratory sicknesses, cold, cough (L)
Brugmansia candida Pers.	Solanaceae	Florifundia	02-0005	Rheumatism, muscle pains (L)
Buddleia americana L.	Scrophulariaceae	Salvia santa	02-0010	Headache, body ache, gastrointestinal ailments (L)
Buddleia davidii Franch.	Scrophulariaceae	Hoja de lanza	02-0013	Asthma (AP)
Bursera simaruba (L.) Sarg.	Burseraceae	Palo de jiote	03-0016	Fever (B)
Byrsonima crassifolia (L.) Kunth	Malpighiaceae	Nance	03-0013	Cough (B)
Capsicum annuum L.	Solanaceae	Chiltepe	616923	Dizziness, faintness of body (AP)
Carica papaya L.	Caricaceae	Papaya	02-0008	Parasites, kidney stones (L)
Cecropia peltata L.	Urticaceae	Guarumo	03-0006	Colic (B)
Cissus verticillata (L.) Nicolson & C.E. Jarvis	Vitaceae	Tobardillo	05-0002	Flu, cold, fever (AP)
Citrus aurantiifolia (Christm.) Swingle	Rutaceae	Limon criollo	03-0015	Tonsillitis, cold, influenza, fever (L)
Citrus aurantium L.	Rutaceae	Naranja agria	03-0014	Cold, dysentery, nausea, fever, sore throat, nerves, depression (L)
Citrus limetta Risso	Rutaceae	Lima	03-0027	Cough (L)
Cissampelos pareira L.	Menispermaceae	Alcotán	05-0003	Stomach ache (B)
Clematis dioica L.	Ranunculaceae	Bejuco de cáncer	05-0002	Wounds (AP)
Cochlospermum vitifolium (Willd.) Spreng.	Bixaceae	Tecomasuche	03-0023	Hepatitis, induce birth (B)
Coriandrum sativum L.	Apiaceae	Cilantro	01-0006	Constipation (children), colic, gas (L)
Cornutia pyramidata L.*	Lamiaceae	Hierba del sope	03-0028	(L)
Crescentia alata Kunth	Bignoniaceae	Morro	03-0012	Respiratory disease, fever, weight loss (B)
Crotalaria longirostrata Hook. & Arn.	Fabaceae	Chipilin	01-0004	Dizziness, faintness (L)
Cupressus lusitanica Mill.	Cupressaceae	Ciprés	03-0024	Cough (L)
Cymbopogon citratus (DC.) Stapf.	Poaceae	Te de limón	01-0028	Bronchitis, asthma, fever (L)
Dianthus caryophyllus L.	Caryophyllaceae	Clavel	02-0004	Gastritis (inflammation of stomach lining), cough (L)
Dyssodia montana (Benth.) A. Gray	Asteraceae	Valeriana	616911	Anxiety (L)
Elephantopus spicatus Juss. ex Aubl.	Asteraceae	Oreja de coche	616922	Fever, malaria, anemia (AP)
Erythrina berteroana Urb.	Fabaceae	Palo de pito	03-0017	Insomnia, induce birth (B)
Eucalyptus globulus Labill.	Myrtaceae	Eucalipto	03-0005	Cough, muscle pain, ear infection, decongestant (L)
Eugenia jambos L.	Myrtaceae	Manzano	03-0030	Cough (L)
Eupatorium semialatum Benth.	Asteraceae	Venadillo	02-0014	Stomach ache, diarrhea (AP)
Gliricidia sepium (Jacq.) Kunth ex Walp.	Fabaceae	Madrecacao	03-0008	Itching (L)
Guazuma ulmifolia Lam.	Malvaceae	Caulote	03-0003	Stomach ache, intestinal infection, clean urinary tract and kidneys (B)
Hamelia patens Jacq.	Rubiaceae	Coloradillo	02-0003	Rheumatism (L)
Ilex aquifolium L.	Aquifoliaceae	Trueno	02-0009	Cough, fever (L)
Jatropha curcas L.	Euphorbiaceae	Pinon	03-0019	Kidney and intestinal problems, heartburn (L)
Jussiaea decurrens (Walter) DC.	Onagraceae	Clavito	01-0008	Kidney stones, clean urinary tract (AP)

Table 1 cont'd.

Liguidambar styraciflua L.	Altingiaceae	Liquidambar	03-0026	Dementia, venereal diseases (L)
Lochnera rosea (L.) Rchb. ex Endl.	Apocynaceae	Chula blanca	01-0005	Bath children with leaves, flower cough syrup, respiratory disease (L)
Lysiloma divaricatum (Jacq.) J.F. Macbr.	Fabaceae	Quebracho	03-0020	Reduce inflammation, healing agent (gums) (B)
Mangifera indica L.	Anacardiaceae	Mango	03-0009	Stomach ache, dysentery, rheumatism (L)
Melia azedarach L.	Meliaceae	Paraiso	03-0017	Fever, rash (L)
Mimosa albida Humb. & Bonpl. ex Willd.*	Leguminosae	Dormilona	616912	(L)
Neurolena lobata (L.) Cass.	Asteraceae	Tres puntas	01-0020	Stomach ache, cough, body ache (AP)
Pelargonium hortorum L.H. Bailey	Geraniaceae	Geranio	01-0010	Relieves spasms, fever, malaria, swollen tonsils, body aches (AP)
Petiveria alliacea L.	Phytolaccaceae	Apacín	01-0002	Dementia, fever, nasal congestion, gas (L)
Pinus oocarpa Schiede ex Schltdl.	Pinaceae	Pino	03-0018	Bronchial asthma (L)
Piper auritum Kunth	Piperaceae	Santa Maria	616920	Gastritis (L)
Plantago major L.	Plantaginaceae	Llanten	616918	Cold, diarrhea, cough, parasites (L)
Pluchea odorata (L.) Cass.	Asteraceae	Siguapacte	02-0011	Headache, cold, rheumatism, body ache (L)
Psidium guajava L.	Myrtaceae	Guayabo	03-0007	Cough (B)
Punica granatum L.	Lythraceae	Granado	02-0006	Diarrhea (B)
Quercus acatenangensis Trel.	Fagaceae	Encino	03-0004	Heal sores, gum inflammations (B)
Quercus benthamii A. DC.	Fabaceae	Roble	03-0025	Cough (B)
Ruta chalepensis L.	Rutaceae	Ruda	01-0016	Conjunctivitis, depression (AP)
Sambucus mexicana Presl. ex DC.	Adoxaceae	Sauco	03-0021	Fever, cough (L)
Sansevieria trifasciata Prain	Dracaenaceae	Curarina	01-0009	Venomous snake bites (poultice), fever (L)
Solanum esculentum Dunal	Solanaceae	Tomate	01-0022	Pimples, boils, burns (L)
Solanum americanum Mill.	Solanaceae	Hierba mora	01-0013	Weakness in body, diabetes, fever (L)
Stevia connata Lag.	Asteraceae	Guapillo	01-0027	Stomach ache, fertility (R)
Tabebuia rosea (Bertol.) A. DC.	Bignoniaceae	Matilisguate	03-0011	Stomach ache, diabetes (B)
Tagetes erecta L.	Asteraceae	Flor de muerto	01-0019	Fever (AP)
Tecoma stans (L.) Juss. ex Kunth	Bignoniaceae	Chacté	02-0001	Cough, dengue hemorrhagic fever, diabetes (excessive consumption harms vision) (L)
Teloxys ambrosioides (L.) W.A. Weber	Amaranthaceae	Apasote	01-0003	Clean the liver, disinfecting wounds (AP)
Tridax procumbens L.	Asteraceae	Hierba del toro	01-0014	Cleaning blood, anemia (AP)
Verbena litoralis Kunth	Verbenaceae	Verbena	01-0024	Diarrhea, stomach ache (AP)
Vernonia leiocarpa DC.	Asteraceae	Suquinay	03-0022	Stomach ache, nausea, diarrhea, wounds (L)
Yucca elephantipes Regel ex Trel.	Alocaceae	Izote	02-0007	Fever (L)
Zanthoxylum culantrillo Kunth	Rutaceae	Uña de gato	01-0016	Nerves, tremors (B)
Zebrina pendula Schnizl.	Commelinaceae	Sangre de pollo	01-0021	Stomach ache, body ache (AP)
Zingiber officinale Roscoe	Zingiberaceae	Jengibre	01-0025	Diarrhea, stomach ache (R)

*Medicinal use not clearly defined at time of collection. [#]Voucher numbers beginning with "61" indicate specimen located at the BYU herbarium (BRY). [†]Tissue extracted: L = leaf; AP = aerial portion; B = Bark; R = root or rhizomes; PS = pod and seed.

Minimum inhibitory concentrations (MICs)

For plant extracts that were inhibitory at 60% or greater (Table 4) in the microbial inhibition assay, MICs were determined using a microwell dilution bioassay (Donaldson et al., 2005). Concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml were tested in triplicate against the microbes. The MIC was determined as the lowest concentration of plant extract at which no reduction of p- iodonitro-

Table 2. Percent inhibition of acetone (A) and methanol (M) extracts from medicinal plant species against cancer cell lines and a non-cancerous Vero control. Values*,# (n = 3) reported as mean ± sd.

Plant species	Percent inhibition (200 µg/ml)									
	Breast		HeLa		Skin		Tongue		Vero	
	A	M	A	M	A	M	A	M	A	M
Acalypha guatemalensis	0	0	0	0	0	0	0	0	0	0
Anacardium occidentale	0	2 ±1	30±3	0	0	2±1	3±2	0	17±8	5±3
Baccharis latifolia	12 ±2	0	39±1	0	**82±3**	43±7	**89±2**	**87±6**	27±1	16±3
Bixa orellana	0	0	3±2	0	0	2±1	0	0	0	3±2
Bougainvillea glabra	0	0	0	0	0	0	0	6±1	0	0
Brugmansia candida	0	0	0	0	0	0	0	2±1	0	0
Buddleia americana	0	3 ±1	0	0	0	3±2	0	4±1	0	0
Buddleia davidii	0	0	0	0	0	0	4±3	0	0	0
Bursera simaruba	**85±13**	**86±3**	**85±1**	**74±7**	5±3	0	0	4±1	0	0
Byrsonima crassifolia	**98±1**	**98±1**	**90±2**	**70±18**	27±5	10±3	13±1	15±5	31±8	29±1
Capsicum annuum	0	0	6±1	0	0	0	0	0	0	0
Carica papaya	0	0	0	0	0	0	0	0	0	0
Cecropia peltata	0	0	0	0	0	0	0	0	0	0
Cissampelos pareira	0	0	0	0	0	0	0	0	0	0
Cissus verticillata	0	0	0	0	0	22±5	0	0	24±4	9±9
Citrus aurantiifolia	0	0	0	0	0	0	0	0	0	0
Citrus aurantium	0	0	0	0	8±4	2±1	0	0	0	0
Citrus limetta	0	0	0	0	0	0	13±6	5±1	0	0
Clematis dioica	3±1	0	0	0	0	0	0	0	0	0
Cochlospermum vitifolium	0	8±5	13±6	4±2	0	0	0	0	14±3	0
Coriandrum sativum	0	0	0	0	0	0	0	0	13±8	0
Cornutia pyramidata	13±2	0	0	0	0	0	0	3±2	0	11±6
Crescentia alata	0	0	13±6	9±6	0	0	0	0	0	0
Crotalaria longirostrata	7±3	0	0	0	0	0	0	13±3	15±2	0
Cupressus lasitanica	0	0	**64±7**	0	0	0	0	4±2	0	0
Cymbopogon citratus	0	0	0	0	0	0	5±2	0	0	0
Dianthus caryophyllus	0	0	0	0	0	0	9±6	0	2±2	2±8
Dyssodia montana	0	0	0	0	0	0	0	0	0	11±5
Elephantopus spicatus	0	0	0	0	**66±3**	20±1	16±4	4±2	48±2	0
Erythrina berteroana	0	7±3	0	0	0	0	0	0	0	0
Eucalyptus globulus	8±4	0	8±2	0	0	0	0	0	0	0
Eugenia jambos	0	0	0	0	0	0	0	0	0	3±2
Eupatorium semialatum	18±4	0	8±1	3±1	**95±2**	50±3	45±8	22±2	13±1	8±5
Gliricidia sepium	0	6±3	0	0	0	0	0	0	0	0
Guazuma ulmifolia	**99±1**	7±2	**95±1**	28±11	0	0	0	0	6±1	0

Table 2. Contd.

Hamelia patens	0	0	8±4	11±6	7±4	7±1	0	0	0	0
Ilex aquifolium	0	0	0	0	0	0	0	0	0	0
Jussiaea decurrens	0	0	0	6±3	0	0	5±1	0	0	0
Liquidambar styraciflua	0	0	4±1	0	0	0	0	0	0	0
Lochnera rosea	9 ±3	**69±1**	0	0	**60±2**	**60±2**	37±6	23±3	0	0
Lysiloma divaricata	0	0	10±3	17±4	0	0	0	0	5±2	0
Mangifera indica	20±1	17±3	0	0	0	0	0	0	0	0
Melia azedarach	0	0	0	0	11±2	11±3	13±1	14±3	0	5±1
Mimosa albida	9 ±1	0	0	3±1	0	0	0	0	0	0
Neurolaena lobata	0	0	57±2	17±4	46±1	18±3	30±2	23±8	23±2	0
Pelargonium hortorum	0	0	12±2	0	0	4±2	0	0	11±4	0
Petiveria alliacea	0	0	0	0	0	0	0	2±1	0	0
Pinus oocarpa	0	0	0	0	0	0	0	0	0	0
Piper auritum	0	0	0	0	0	0	4±2	0	0	0
Plantago major	0	0	0	0	0	0	0	0	0	13±5
Pluchea odorata	0	0	0	0	0	0	0	0	0	0
Psidium guajava	**83±3**	0	36±3	0	0	0	52±17	12±2	24±1	8±5
Punica granatum	0	0	0	0	0	0	0	0	5±1	0
Quercus acatenangensis	**94±4**	26±7	**95±1**	**83±3**	0	0	0	0	9±4	14±1
Quercus benthamii	0	0	52±14	29±5	6±1	0	0	0	0	4±1
Ruta chalepensis	0	0	0	0	4±1	0	0	0	0	0
Sambucus mexicana	0	0	0	0	0	0	0	3±1	0	0
Sansevieria trifasciata	0	0	0	0	0	0	0	0	0	0
Solanum esculentum	3 ±1	4±2	**92±1**	**81±6**	**75±2**	**91±2**	**95±2**	**98±1**	66±2	91±2
Solanum americanum	42±12	33±2	**85±3**	**92±1**	0	55±8	0	**99±1**	47±2	76±3
Stevia connata	0	0	0	9±2	0	0	0	0	0	0
Tabebuia rosea	0	0	0	0	0	0	0	0	0	0
Tagetes erecta	0	0	0	25±8	0	0	0	0	0	0
Tecoma stans	0	0	0	0	0	0	0	0	0	0
Teloxys ambrosioides	0	0	0	0	0	0	0	0	0	0
Tridax procumbens	0	0	0	0	0	0	0	0	0	0
Verbena litoralis	0	0	6±4	0	0	0	0	0	0	0
Vernonia leiocarpa	0	0	0	0	0	0	0	0	0	0
Yucca elephantipes	0	0	0	0	0	0	0	0	0	0
Zanthoxylum culantrillo	0	0	0	0	0	0	0	0	0	0
Zebrina pendula	**95±1**	0	0	0	0	0	0	0	0	0
Zingiber officinale	0	0	0	0	0	0	0	6±3	0	5±1

*Inhibition values in bold indicate extracts that were considered active based on cancer inhibition of 60% or greater. [#]Values over 60% or greater inhibition significantly different from controls at $P \leq 0.05$.

Table 3. Half-maximum inhibitory concentrations (IC_{50}) for cancer cell lines and half-maximum cytotoxicity concentrations (CC_{50}) for the Vero cell line when subjected to plant extracts. Values reported as mean ± sd.

Cancer cell line/plant species	IC_{50} (µg/ml)		CC_{50} (µg/ml)	
	A	M	A	M
Breast				
Bursera simaruba	113±38	116±14	>800	>800
Byrsonima crassifolia	103±33	52±9	>800	>800
Guazuma ulmifolia	67±3	-	>800	-
Lochnera rosea	-	>200	-	>800
Psidium guajava	115±11	-	105 ±7	-
Quercus acatenangensis	86±3	-	>800	-
Zebrina pendula	>200	-	>800	-
HeLa				
Bursera simaruba	148±14	170±18	>800	>800
Byrsonima crassifolia	86±3	72±3	>800	>800
Cupressus lusitanica	>200	-	>800	-
Guazumaulmifolia	68±6	-	>800	-
Quercus acatenangensis	86±4	77±4	>800	>800
Solanum esculentum	60±4	33±6	75±31	20±2
Solanumamericanum	>200	195±4	450±13	127±2
Skin				
Baccharis latifolia	119±60	-	91±2	-
Elephantopus spicatus	>200	-	255±68	-
Eupatorium semialatum	>200	-	131±28	-
Lochnera rosea	>200	>200	>800	>800
Solanum esculentum	78±2	47±18	75±31	20±2
Solanum americanum	139±9	-	450±13	-
Tongue				
Baccharis latifolia	75±4	>200	91±2	300±23
Solanum esculentum	*	36±2	-	20±2
Solanum americanum	-	171±3	-	127±2

*The acetone extract of *S. esculentum* was not tested against tongue cancer cells in the NR assay due to lack of extract.

tetrazolium violet dye (Sigma-Aldrich) was observed (Mann and Markham, 1998). MICs were not calculated for *S. mutans* and *L. acidophilus* due to irregular growth and clumping.

Data analysis

Data were coded by species and fraction and statistical significance ($P \leq 0.05$) between control versus inhibition values was determined by analysis of variance (ANOVA) (SPSS, 2011 results from the 200 µg/ml concentration used against cancer cell lines) and the 1000 µg/ml concentration used against the microbes are the only results reported (Tables 2 and 4). This is because these concentrations yielded the maximum number of active plant species that might be considered for future studies. Consequently, any extract showing greater than 60% inhibition for the acetone or methanol extracts at the 200 µg/ml level for any cancer cell line, and at the 1000 µg/ml for any microbial species, was considered active and worthy of neutral red or MIC analysis. An additional criterion was that if the inhibition level of a cancer cell line was two to three times that of the vero line then those extracts also were considered active.

RESULTS

Sulforhodamine B assay for inhibition and cytotoxicity

Of the 73 species screened, extracts from 13 (17.8%) species resulted in an inhibition level of 60% or greater against at least one cancer cell line (Table 2). For the breast cancer cell line, four species had active acetone extracts, one had an active methanol extract, and two species had active acetone and methanol extracts. The acetone extract from two species and the acetone and methanol extracts from five species were

Table 4. Percent inhibition of acetone (A) and methanol (M) extracts from medicinal plant species against microbes. Values[#,†] reported as mean ± sd.

Plant species	Percent Inhibition (1000 µg/ml)							
	S. aureus		*S. mutans*		*E. coli*		*C. albicans*	
	A	M	A	M	A	M	A	M
Acalypha guatemalensis	0	0	0	0	0	0	0	0
Anacardium occidentale	**64±5**	18±5	0	23±3	0	0	0	0
Baccharis latifolia	25±3	0	4±2	4±2	0	0	0	0
Bixa orellana	28±8	27±1	0	0	0	0	0	0
Bougainvillea glabra	0	0	5±1	0	0	0	0	0
Brugmansia candida	0	0		0	0	0	0	0
Buddleia americana	5±2	0	0	0	0	0	0	0
Buddleia davidii	0	0	0	0	0	0	0	0
Bursera simaruba	8±5	8±3	0	0	0	0	0	0
Byrsonima crassifolia	32±8	0	17±1	16±2	6±1	0	0	0
Capsicum annuum	0	0	0	0	0	0	0	0
Carica papaya	0	0	0	0	0	0	0	0
Cecropia peltata	0	0	14±2	0	0	0	0	0
Cissampelos pareira	0	0	0	0	0	0	0	0
Cissus verticillata	0	0	31±4	0	0	0	34±3	54±6
Citrus aurantiifolia	0	0	0	0	11±3	0	0	0
Citrus aurantium	0	0	0	0	0	0	0	0
Citrus limetto	0	0	0	0	0	0	0	0
Clematis dioica	0	0	9±1	13±2	0	0	0	0
Cochlospermum vitifolium	39±2	10±2	0	0	4±1	0	0	0
Coriandrium sativum	0	0	0	0	0	0	0	0
Cornutia pyramidata	0	0	39±2	43±1	0	0	0	0
Crescentia alata	14±1	0	0	0	0	0	0	0
Crotalaria longirostrata	0	0	52±1	52±1	0	0	0	0
Cupressus lusitanica	0	5±1	8±3	8±1	0	0	0	0
Cymbopogon citratus	0	0	5±2	0	0	0	0	0
Dianthus caryophyllus	0	0	0	0	0	0	0	0
Dyssodia montana	0	7±4	0	0	0	0	0	0
Elephantopus spicatus	7±4	0	6±2	0	0	0	0	0
Erythrina berteroana	0	0	0	0	3±1	5±1	49±9	42±5
Eucalyptus globulus	**61±3**	0	54±1	0	17±2	0	51±8	17±2
Eugenia jambos	23±5	22±7	0	-	29±5	0	0	0
Eupatorium semialatum	0	0	0	0	0	0	45±3	41±6
Gliricidia sepium	0	0	0	0	0	0	0	0
Guazuma ulmifolia	30±8	0	0	0	3±1	0	0	0
Hamelia patens	0	0	2±1	5±1	0	0	0	0
Ilex aquifolium	0	0	0	0	0	0	0	0
Jatropha curcas	0	0	0	0	9±6	0	0	0
Jussiaea decurrens	39±3	0	56±5	0	0	0	0	0
Liquidambar styraciflua	**65±3**	57±6	24±1	0	14±2	4±1	0	0
Lochnera rosea	0	0	6±2	11±1	0	0	0	0
Lysiloma divaricatum	32±3	28±5	24±3	20±6	0	0	0	0
Mangifera indica	20±3	12±2	0	0	17±1	17±4	0	0
Melia azedarach	0	0	0	0	0	0	0	0
Mimosa albida	36±12	27±8	38±2	0	14±1	0	0	0
Neurolaena lobata	28±3	4±1	0	0	0	0	0	0
Pelargonium hortorum[*]	57±4	27±5	26±2	13±3	26±2	0	0	0
Petiveria alliacea	0	0	32±2	32±1	0	0	0	0

Table 4. Contd.

Pinus maximinoi	0	0	0	0	0	0	0	0
Piper auritum	0	0	0	0	0	0	5±2	49±5
Plantago major	0	0	9±1	0	0	3±1	0	0
Pluchea odorata	0	0	0	0	0	0	0	0
Psidium guajava	**75±4**	40±1	**64±10**	12±4	45±1	34±2	0	0
Punica granatum	36±4	0	36±10	2±1	16±2	0	0	0
Quercus acatenangensis	46±3	35±5	14±10	3±11	6±5	0	0	0
Querus benthamii	54±3	47±1	0	7±3	25±3	15±6	0	0
Ruta chalepensis	0	0	0	0	0	0	0	0
Sambucus mexicana	5±2	5±1	0	0	0	0	0	0
Sansevieria trifasciata	11±4	20±4	0	26±8	0	0	0	0
Solanum esculentum	0	0	10±5	7±3	0	0	0	0
Solanum americanum	0	0	7±1	0	0	0	0	48±8
Stevia connata	0	0	0	0	0	0	0	0
Tabebuia rosea	0	0	3±1	0	0	0	6±1	0
Tagetes erecta	0	0	0	0	0	0	0	0
Tecoma stans	0	0	0	3±1	0	0	0	0
Teloxys ambrosioides	0	0	0	0	0	0	0	0
Tridax procumbens	0	0	0	0	0	0	0	0
Verbena litoralis	0	0	0	0	0	0	0	0
Vernonia leiocarpa	0	0	0	5±2	0	0	0	0
Yucca elephantipes	0	0	0	0	0	0	0	0
Zanthoxylum culantrillo	6±4	-	38±2	36±2	4±1	0	0	0
Zebrina pendula	12±1	0	5±1	11±2	0	0	0	0
Zingiber officinale	0	0	0	9±2	0	0	0	39±9

[#]Inhibition values in bold indicate extracts that were considered active based on microbe inhibition of 60% or greater. [†] Values 60% or greater inhibition significantly different from controls at P ≤ 0.05.*P. hortorum* was the only species inhibitory to *Lactobacillus acidophilus* at 68% inhibition (acetone extract).

active against the HeLa (cervical) cell line. The acetone extracts from four species, the methanol extract from one species, and the acetone and methanol extracts from one other species were active against the skin cancer cell line. Against the tongue cell line, one species had an active methanol extract and two species had active acetone and methanol extracts (Table 2). A few extracts also showed specificity against a single cell line. These were the acetone extracts of *Psidium guajava* L. (Myrtaceae) and *Zebrina pendula* Schnizl. (Commelinaceae) which were active against the breast cell line, *Cupressus lusitanica* Mill. (Cupressaceae) was active against the HeLa line, and *Elephantopus spicatus* Juss. ex Aubl. (Asteraceae) and *Eupatorium semialatum* Benth. (Asteraceae) were active against the skin cell line (Table 2). It is noteworthy that 10 of the 13 species considered active against one or more cancer cell lines were not deemed cytotoxic against the vero cell line. The three species yielding extracts toxic to the vero cell line based on either a 60% or greater level of inhibition or more than a three-fold difference between the level of inhibition against the vero line versus that of a cancer cell line were *E. spicatus*, *S. esculentum*, and *S. americanum* (Table 2).

Neutral red (NR) assay for inhibition and cytotoxicity

The acetone extracts from *Guazuma ulmifolia* Lam. (Malvaceae) and *Quercus acatenangensis* Trel. (Fagaceae) were highly inhibitory at low concentrations against breast cancer (IC_{50} < 100 µg/ml) and showed low inhibition at high concentrations against vero cells (CC_{50} > 800 µg/ml) (Table 3). The methanol extract from *Byrsonima crassifolia* (L.) Kunth (Malpighiaceae) also was highly inhibitory at low concentrations against breast cancer with low inhibition at high concentrations against vero cells. Additionally, the acetone and methanol extracts of *Bursera simaruba* (L.) Sarg. (Burseraceae) and the acetone extract of *B. crassifolia* were moderately inhibitory to breast cancer cells (100 µg/ml < IC_{50} < 200 µg/ml) with low inhibition at high concentrations against vero. The acetone extract of *G. ulmifolia* and the acetone and methanol extracts of *B. crassifolia* and *Q. acatenangensis* were highly inhibitory at low concentrations against the HeLa cancer cell line with IC_{50} <100 µg/ml

Table 5. Minimum inhibitory concentrations (MIC) for medicinal plants against microbes.

Plant species	MIC (µg/ml; acetone extract)	
	S. aureus	***C. albicans***
Anacardium occidentale	1000	>1000
Eucalyptus globulus	125	250
Liquidambar styraciflua	250	>1000
Pelargonium hortorum	250	>1000
Psidium guajava	125	>1000

and CC_{50} > 800 µg/ml (Table 3). Acetone and methanol extracts of *B. simaruba* showed moderate inhibition against the HeLa line (100 µg/ml < IC_{50} < 200 µg/ml) but low inhibition at high concentrations against vero. Alternatively, high IC_{50} values and/or low CC_{50} values indicated that *Lochnera rosea* (L.) Rchb. ex Endl. (Apocynaceae), *P. guajava*, *Z. pendula*, *C. lusitanica*, *Baccharis latifolia* (Ruiz and Pav.) Pers. (Asteraceae), *E. semialatum*, *E. spicatus*, *Solanum americanum* L. (Solanaceae), and *S. esculentum* Dunal (Solanaceae) were cytotoxic to the cell lines tested and therefore may not be candidates for future studies unless further fractionation is undertaken.

Microbial inhibition assay

Of the 73 medicinal plant species analyzed for activity against pathogenic microbes, five had inhibition levels at 60% or greater against one or more microbial species (Table 4). Four of the five plant species are found in Table 4, and the fifth species *Pelargonium hortorum* L.H. Bailey (Geraniaceae) yielded an acetone extract that was 68% inhibitory to *L. acidophilus* (see Table 4 footnote). The acetone extracts from four species were active against S. *aureus* and the acetone extract from *P. guajava* was active against *S. mutans*. Some specificity of plant extracts was noted as *Anacardium occidentale* L. (Anacardiaceae), *E. globulus*, and *Liquidambar styraciflua* L. (Altingiaceae) were active against *S. aureus*.

Minimum inhibitory concentrations

The acetone extracts of *P. hortorum* and *L. styraciflua* inhibited the growth of *S. aureus* with a MIC of 250 µg/ml (Table 5). The concentrations of the acetone extracts from *P. guajava* and *E. globulus* required to inhibit growth of *S. aureus* were lower (MIC = 125 µg/ml). The MIC value for the acetone extract of *A. occidentale* for *S. aureus* was 1000 µg/ml indicating limited activity. The MIC value for the acetone extract of *E. globulus* was 250 µg/ml for *C. albicans* (Table 5).

DISCUSSION

Our study and that of Kufer et al. (2005) report how villagers in our respective study areas use medicinal plants for health needs (Table 1). Though there is overlap between what the villagers noted as the common uses and the uses that are published (Cáceres, 2009; Kufer et al., 2005; Comerford, 1996), there are discrepancies. Consequently, it appears that villagers are losing some of the traditional knowledge about the use of medicinal plants and this seems to vary from village to village (Ardon, 2008; Galvez, 2008). Overall, 17 species (23.3%, including *P. hortorum*) were inhibitory to one or more cancer cell lines and/or microbes at 60% inhibition or greater (Tables 2 and 4). Thirteen were inhibitory to one or more cancer cell lines, five species were active against one or more microbes, and *P. guajava* overlapped in activity against one cancer cell line and two microbes (Tables 2 and 4). Based on the criteria for cytotoxicity against the vero cell line outlined in the data analysis section, the ratio of IC_{50}/CC_{50} (Table 3), and MIC values above 250 µg/ml (Table 5), nine of these 17 species would require further fractionation to identify non-toxic but active compounds before further work could be undertaken. These are *A. occidentale* (Table 5), *B. latifolia*, *C. lusitanica*, *E. semialatum*, *E. spicatus*, *L. rosea*, *S. esculentum*, *S. americanum*, and *Z. pendula* (Tables 2 and 3).

P. hortorum, *L. styraciflua*, *P. guajava*, and *E. globulus* yielded low MIC values (≤ 250 µg/ml) against *S. aureus*. *E. globulus* was the only plant active against *C. albicans* (Table 5). At least two of these species produce essential oils which have been implicated as the active compounds responsible for plant extract-induced microbial growth inhibition (Edris, 2007; Gutíerrez et al., 2008). Noteworthy is that the plant tissue used in this study was first extracted with hexane to remove essential oils. The MIC values of 125 µg/ml from the extracts of *E. globulus* and *P. guajava* against *S. aureus* suggests that compounds such as flavonoids (Takahashi et al., 2004) may be active in addition to essential oils commonly found to have antimicrobial activity (Mulyaningsih et al., 2011).

Based on results from this study, *B. simaruba*, *B. crassifolia*, *E. globulus*, *G. ulmifolia* (Tables 2 and 3), *L. styraciflua*, *P. hortorum*, *P. guajava*, and *Q. acatenangensis* (Tables 4 and 5) merit consideration for future study. All of these species are well established as medicinal plants used by rural villagers against a variety of ailments (Table 1) (Cáceres, 2009). *B. crassifolia* also has been linked to neuropharmacological activity (Morales Cifuentes et al., 2001) and antimicrobial activity (Martínez-Vázquez et al., 1999). In this study *B. crassifolia* was active against breast and HeLa cancer cell lines, but not against microbes (Table 4).

P. guajava is well known as a medicinal plant in tropical and subtropical countries where it is used to treat a large numberofailmentsincludinggastrointestinaland respiratory

problems (Gutíerrez et al., 2008; Sanda et al., 2011). Significant activity of extracts and known compounds found in *P. guajava* against *S. aureus* and *E. coli* as well as anti-proliferative activity are noted in these reviews.

Of the 17 species that showed significant levels of inhibition (Tables 2 to 5), seven (41%) are well documented as important medicinal plants (Cáceres, 2009). In our study *P*. *guajava* showed significant activity against *S*. *aureus* and *S*. *mutans* (Table 4). The acetone extract of *P*. *guajava* also had a low MIC value (125 µg/ml) against *S*. *aureus* (Table 5) but little activity was noted for extracts from *G. ulmifolia* against any microbe (Table 4). Also, *A*. *occidentale, B*. *crassifolia*, *P*. *guajava*, and *G*. *ulmifolia* were found to have activity against one or more enterobacteria (Cáceres et al., 1990) and *G. ulmifolia* also was active against two bacteria known to cause dematomucosal diseases (Cáceres et al., 1987). Madureira et al. (2012) showed that a methanol extract from the aerial tissues of *A. occidentale* was significantly inhibitory to *S. aureus* with an MIC of 7.5 µg/ml. In our study, *A. occidentale* significantly inhibited *S. aureus* (Table 4) but the MIC was 1000 µg/ml (Table 5). This discrepancy in MIC may be due to differences in extraction methods and/or in the microbial strain used.

Cáceres et al. (1993a) showed that *B*. *crassifolia* and *P*. *guajava* have activity against *S*. *pyrogenes* and *S*. *aureus*, respectively. *B*. *crassifolia* also was found to have some antifungal activity (Cáceres et al., 1993b). For the 20 species found in Cáceres et al. (1987, 1990) that overlap with this study (Tables 4 and 5), patterns of inactivity or activity are similar against *E*. *coli* even though different strains and methods were used. Similar to the study reported here, Mothana et al. (2011) did not find significant activity from the methanol extract in agar diffusion or MIC assays for *Melia azedarach* L. (Meliaceae) against *S*. *aureus*, *E*. *coli*, or a breast cancer cell line.

Conclusion

Seventeen medicinal plant species were found to be inhibitory to one or more cancer cell lines and/or microbes. However, cytotoxicity to the vero cell line, high IC_{50} values and low CC_{50} values, and high MIC values indicated that nine of these species may not merit further study. The eight species that do merit further research as to their active compounds, mechanism of action, and in animal and clinical studies were *B*. *simaruba*, *B*. *crassifolia*, *E*. *globulus*, *G*. *ulmifolia*, *L*. *styraciflua*, *P*. *guajava*, *P*. *hortorum*, and *Q*. *acatenangensis*.

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