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Activities of Guatemalan medicinal plants against cancer cell lines and selected microbes: Evidence for their conservation

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Medicinal plants are important components in the primary health care of villagers in Guatemala. However, an area often overlooked is the effect of medicinal plants on oral hygiene. Acetone and methanol extracts from 63 medicinal plant species from 31 families were bioassayed against breast, cervical, skin and tongue cancers, and the following microorganisms: *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 against microbes. Based on levels of inhibition by extracts, IC_{50} values, CC_{50} values, and MIC values, seven species (*Bursera simaruba* Sarg., Burseraceae; *Eriobotrya japonica* (Thumb.) Lindl., Rosaceae; *Litsea guatemalensis* Mez, Lauraceae; *Mirabilis jalapa* L., Nyctaginaceae; *Pithecellobium dulce* (Roxb.) Benth., Fabaceae); *Rubus villosus* Thunb., Rosaceae; *Thevetia peruviana* K. Schum., Apocynaceae) were recommended for additional investigation. With regard to oral hygiene four species (*Achillea millefolium* L., Asteraceae; *Crotalaria longirostrata* Hook. and Arn., Fabaceae; *P. dulce*; *Spondias purpurea* L., Anacardiaceae) may merit further fractionation and testing against oral diseases.

Key words: Anticancer, antimicrobial, IC_{50}, CC_{50}, MIC, oral hygiene, Guatemala.

INTRODUCTION

Well documented is the use and value of the earth's medicinal resources with regard to primary health care for the human population. For example, Kingston (2011) and Newman and Cragg (2007) suggest that up to 50% of the drugs now available to treat human diseases are related to natural products. For anticancer, anti-migraine, and other drugs the estimate is well over 50% (Newman and Cragg, 2012; Butler, 2008; McChesney et al., 2007).

However, Newman et al. (2008), Adams and Hawkins (2007), and Chaudhuri (2007) noted that global access to these types of drugs is highly variable. The result is that traditional remedies support the health care of over 65% of the world population (Fabricant and Farnsworth, 2001), and in rural communities the estimate is 75 to 90% (Chivian and Bernstein, 2008; Fowler, 2006), depending on the geographical area.

An additional consideration is that traditional knowledge and the biodiversity that supports that knowledge and the development of new drugs are being lost (Cordell and Colvard, 2012; Strobel et al., 2004). These in combination with the evolution of drug resistance (Lambert et al., 2011) contribute to the increased awareness to conserve these valuable plant resources (Siwach et al., 2013; Kingston, 2011). Another concern regarding the primary health care of people in rural communities worldwide is the lack of information on the role of medicinal plants to improve oral hygiene (Colvard et al., 2006), For example, Kufer et al. (2005) in their study on the use of medicinal plants in the Ch'orto' area in southeastern Guatemala listed about 41 plants that were used to treat gastrointestinal illnesses, 34 species used for fever and pain, 38 for women's remedies, 25 for respiratory illnesses, but only seven for oral health problems. Of these seven, three were used in prevention and all seven were used for toothaches. Rural family members in southeastern Guatemala near Esquipulas who were suffering from toothache or orofacial pain resorted to using nine herbals but no traditional remedies were noted to prevent cavities or other oral cavity diseases (Hunter and Arbona, 1995). Consequently, a need exists to find medicinal plants that have potential to prevent and treat periodontal diseases and other oral health issues.

These concerns are relevant to the health care of villagers in Guatemala and therefore formed the basis for this study. The first objective was to evaluate the *in vitro* growth inhibition of acetone and methanol extracts from 63 plant species against breast, cervical, skin, and tongue cancer cell lines and a non-cancerous line. For those extracts that were inhibitory at 60% or greater IC_{50} and CC_{50} values were determined. Secondly, *in vitro* growth inhibition of these extracts against *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Lactobacillus acidophilus*, and *Candida albicans* were determined. For those active at 60% or greater minimum inhibitory concentrations (MIC) were obtained. All 63 species are noted in Guatemalan health care pharmacopoeias and about half of these species are used for oral health care. Consequently, activity against *Streptococcus mutans*, *Lactobacillus acidophilus*, *Candida albicans* and the tongue cancer cell line was of particular interest due to their association with dental plaque, caries, and other oral cavity health issues (Kleinberg, 2002).

MATERIALS AND METHODS

Plant collection, tissue preparation, cell lines and microbial cultures

Eighteen species were collected from the Museo Odontológico de Guatemala y Jardín Botánico Maya, Guatemala City, Guatemala, 20 species from Colección y Huerto Productivo de Plantas Medicinales, Facultad de Agronomía, Guatemala City, and 25 from the communities of Olopa and San Juan Ermita in southeastern Guatemala. Aids in identifying species other than vouchers and digital pictures were the *Vademecum National de Plantas Medicinales* (Cáceres, 2009), the guide to medicinal plants by Arevalo and Dieseldorff (2005), and a species list for the Museo Odontologico de Guatemala y Jardin Botánico Maya. Voucher specimens are located in the herbaria at the Centro Universitario de Oriente, Universidad de San Carlos de Guatemala, Chiquimula, Guatemala (CUNORI) and at Brigham Young University (BRY), Provo, UT. Each sample from the 63 species analyzed consisted of tissue (Table 1) collected from three or more individuals that was mixed, then bagged, labelled, and stored at -80^{o} C (Isotemp Basic, Thermo Electron Corporation, Asheville, NC USA) at BYU. Acetone and methanol extracts derived from five grams of plant tissue were eventually dissolved in double-distilled water at a final concentration of 8 mg/ml. 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-2095,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). *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. Methods for culturing cancer cell lines, the non-cancerous cell line, and microbes are described by Cates et al. (2013).

Sulforhodamine B assay and neutral red (NR) assay

The sulforhodamine B assay used to determine the level of inhibition of extracts against cancer cell lines followed Skehan et al. (1990) and Donaldson et al. (2004) as described by Cates et al. (2013). Inhibition activity against cell lines was determined in triplicate at 200, 100, and 50 µg/ml of extract. Results in Table 2 are reported only for the 200 µg/ml concentration. The NR assay followed Putnam et al. (2002) and was used on all extracts that showed 60% or greater inhibition in the sulforhodamine assay. Serial dilutions of 200, 100, 50, 25, 12.5 and 6.25 µg/ml of each plant extract were run in triplicate against each cell line (Cates et al., 2013). 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

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Table 1. Scientific names, common names, tissue collected, and use of medicinal plants.

Scientific name	Family	Common name	Tissue extracted	Medicinal use
Acacia farnesiana (L.) Willd.	Leguminosae	Subin	Leaves	Vaginal bleeding, fertility, after childbirth, cold*
Acalypha guatemalensis Pax & K. Hoffm.	Euphorbiaceae	Hierba de cancer	Leaves	Gum disease, tooth ache, cancer
Achillea millefolium L.	Asteraceae	Milenrama	Aerial portion	Fever, colds, dysenteria
Allium sativum L.	Liliaceae	Ajo	Bulb	Digestion disorders, respiratory diseases
Anethum graveolens L.	Apiaceae	Hinojo	Leaves	Diarrhea, after birth antiseptic, stomach pain
Anthemis oppositifolia Lam.#	Asteraceae	Ixmaramac	Leaves	Anesthetic
Arnica montana L.†	Asteraceae	Arnica	Aerial portion	
Asclepias curassavica L.	Apocynaceae	Cuajatinta	Leaves	Fever
Baccharis trinervis Pers.	Asteraceae	Corrimiento	Leaves	Anagelsic
Bourreria huanita (Lex.) Hemsl.	Boraginaceae	Esquisuchil	Leaves	Fever, cold
Brosimum alicastrum Sw.	Moraceae	Ramon (Ujuxte)	Green fruit	Cough, sore throat
Brugmansia candida Pers.	Solanaceae	Florifundia	Leaves	Tooth ache pain, sleep agent
Bursera simaruba (L.) Sarg.	Burseraceae	Palo de jiote	Leaves	Wounds, insect bites, stings
Casimiroa edulis La Llave & Lex.	Rutaceae	Matasano	Roots	Birthing accelerant
Cedrela odorata L.	Meliaceae	Cedro	Bark (inner)	Tooth pain, birthing accelerant
Cinnamomum zeylanicum Blume	Lauraceae	Canela	Leaves	Fever, headache, cold, diarrhea
Citrus sinensis (L.) Osbeck	Rutaceae	Naranja	Leaves	Anxiety, depression
Coffea arabica L.	Rubiaceae	Café	Leaves	Dizziness
Costus pictus D. Don†	Costaceae	Cana de cristo	Leaves	
Crotalaria longirostrata Hook & Arn.	Fabaceae	Chipilin	Leaves	Sedative, anemia, insomnia
Cupressus lusitanica Mill.	Cupressaceae	Cipres	Needles	Cough
Equisetum arvense L.	Equisetaceae	Oreja de coche	Aerial portion	Gripe
Eriobotrya japonica (Thumb.) Lindl.	Rosaceae	Nispero	Green fruit	Tooth pain, gum inflammation
Euphorbia lancifolia Schldlt.	Euphorbiaceae	Ixbut	Leaves	Lactation stimulate, impotence, cold
Fleischmannia pycnocephala (Less,) R. M. King and H. Rob.	Asteraceae	Violeta‡	Aerial portion	Respiratory problems
Hibiscus sabdariffa L.	Malvaceae	Rosa de Jamaica	Leaves	Intestinal distress, chicken pox
Ixora coccinea L.	Rubiaceae	Coralillo	Leaves	Muscle relaxant
Jatropha curcas L.	Euphorbiaceae	Pinon	Leaves	Kidney and intestinal problems, heartburn, inflamed gums
Latana camara L.	Verbenaceae	Cinco negritos	Leaves	Female hemorrhaging, discharge
Lippia dulcis Trevir.	Verbenaceae	Orosus	Aerial portion	Bronchitis
Lippia graveolens Kunth	Verbenaceae	Oregano	Aerial portion	Pain from tooth ache, spice
Litsea guatemalensis Mez	Lauraceae	Laurel	Leaves	Gastrointestinal problems, colic, swelling
Mirabilis jalapa L.	Nyctaginaceae	Flor de maravilla	Aerial portion	Cold, influenza, diarrhea
Murraya paniculata (L.) Jack	Rutaceae	Limonaria	Leaves	Relieve tooth ache pain
Nicotiana tabacum L.	Solanaceae	Tabaco	Leaves	Tooth ache
Ocimum basilicum L.	Lamiaceae	Albahaca morada	Aerial portion	Gastrointestinal spasm, migraine headaches
Ocimum micranthum Willd.	Lamiaceae	Albahaca del monte	Aerial portion	Stomach ache
Origanum vulgare L.	Lamiaceae	Oregano de Castillo	Aerial portion	Menstruation
Passiflora lingularis Juss.	Heliconiaceae	Granadilla	Leaves	Anxiety, tooth ache pain
Persea americana Mill	Lauraceae	Aguacate	Leaves	Tooth ache, rheumatism, cough
Petiveria alliacea L.	Phytolacaceae	Apacin	Leaves	Fever, nasal congestion, gastritis, diarrhea
Pimenta dioica (L.) Merr.	Myrtaceae	Peinata	Leaves	Anesthetic, stomach pain
Piper auritum Kunth	Piperaceae	Santa Maria	Leaves	Cancer
Pithecellobium dulce (Roxb.) Benth.	Fabaceae	Shaguay	Bark	Kidney stones
Priva lappulacea (L.) Pers.	Verbenaceae	Mozotillo	Leaves	Kidney disease
Prunus persica (L.) Batsch	Rosaceae	Duranzo	Leaves	Cold, cough, eating
Punica granatum L.	Lythraceae	Granado	Leaves	Tooth ache, diarrhea
Rauvolfia tetraphylla L.	Apocynaceae	Chalchupa	Leaves	Hypertension
Rhus terebinthifolia Schlech &Cham	Anacardiaceae	Sal de venado	Leaves	Tooth ache pain, gum disease
Rosmarinus officinalis L.	Lamiaceae	Romero	Aerial portion	Colic, bronchitis, anemia
Rubus villosus Lasch.	Rosaceae	Sarzamora	Leaves	Cold, cough, influenza, diarrhea, parasites
Senna occidentalis L.	Fabaceae	Frijolillo	Leaves	Dental disease
Solanum torvum Sw.	Solanaceae	Chichita	Leaves	Bronchitis, cold, diarrhea
Solanum umbellatum Miller#	Solanaceae	Tabaquillo	Leaves	Cleaning powder for teeth, tooth ache
Spondias purpurea L.	Anacardiaceae	Jocote	Leaves	Astringent, diarrhea, dysentery

Table 1 cont'd

Stigmaphyllon ellipticum A. Juss.	Malpighiaceae	Contra hierba	Leaves	Snake bites, tooth ache
Tagetes filifolia Lag.	Asteraceae	Anis de monte	Leaves	Stomach ache, diarrhea*
Tagetes lucida Cav.	Asteraceae	Pericon	Leaves	Abdominal and menstrual pain
Taraxacum officinale F. H. Wigg.	Asteraceae	Amargon	Leaves	Hepatic and urinary disorders
Thevetia peruviana Merr.	Apocynaceae	Quiebra la muela	Leaves	Paste applied to cavity for tooth removal
Thymus vulgaris L.	Lamiaceae	Tomillo	Aerial portion	Respiratory infections, bronchitis, cough
Tridax procumbens L.	Asteraceae	Hierba del toro	Aerial portion	Hemorrhage
Vetiveria zizanioides (L.) Nash†	Poaceae	Vetiver grass (Valeriana)‡	Leaves	

*Information from Kufer et al. (2005).
#*Anthemis oppositifolia* and *Solanum umbellatum* were not analyzed for activity against microbes due to lack of tissue.
†Medicinal use not clearly defined at time of collection.
‡Local villagers referred to *V. zizanioides* as Valeriana and *F. pycnocephala* as violet.

(IC_{50}) and half-maximum cytotoxicity concentrations (CC_{50}). The IC_{50} and CC_{50} values were obtained using dosage response curves.

Microbial inhibition assay and minimum inhibitory concentrations (MIC)

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 only percent inhibition at the 1000 µg/ml concentration was reported (Table 4). For plant extracts that were inhibitory at 60% or greater (Table 4) MICs were determined using a microwell dilution bioassay. Concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/ml were tested in triplicate against the microbes. The MIC was defined as the lowest concentration of extract at which no reduction of *p*-iodonitro-tetrazolium violet dye (Sigma-Aldrich) was observed. MICs were not calculated for *S. mutans* and *L. acidophilus* due to irregular growth and clumping. Details of these two assays are found in Cates et al. (2013).

Data analysis

Data were coded by species and fraction and statistical significance ($P \leq 0.001$) between control vs. inhibition values were determined by ANOVA (R Core Team, 2013). 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. 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 were considered active.

RESULTS

Sulphorhodamine inhibition and cytotoxicity to Vero cells

Eight (12.7%) of the 63 species analyzed showed activity against one or more of the cancer cell lines (Table 2). The acetone extracts of *Persea americana* Mill. (Lauraceae) and *Pithecellobium dulce* (Roxb.) Benth. (Fabaceae) were active against breast cancer cells (97% and 73% inhibition, respectively). The methanol extract (96%) of *Bursera simaruba* (L.) Sarg. (Burseraceae) and the acetone and methanol extracts (70 and 60%, respectively) of *Litsea guatemalensis* Mez (Lauraceae) were also active against this cell line. The acetone extract (94%) from *P. americana* and the methanol extract (75%) of *Cedrela odorata* L. (Meliaceae) were active against the HeLa line (Table 2). Acetone and methanol (68 and 69%, respectively) extracts from *Solanum umbellatum* Miller (Solanaceae) and *Thevetia peruviana* Merr. (Apocynaceae) (60 and 68%, respectively) also were active against this line. C*rotolaria longirostrata* Hook. and Arn. (Fabaceae) produced an acetone extract that was active against skin and tongue cell lines (62% and 61% inhibition, respectively), and the methanol extract (62%) of *T. peruviana* was active against the skin cancer cell line (Table 2). However, the acetone extracts from *C. longirostrata, P. dulce* and the acetone and methanol extracts from *T. peruviana* showed cytotoxic effects against the non-cancerous Vero cell line.

Neutral red (NR) assay for inhibition and cytotoxicity

The methanol extract from *B. simaruba* and the acetone extract from *T. peruviana* were highly inhibitory at low concentrations (IC_{50} = 75 µg/ml and 30 µg/ml, respectively) against the breast and HeLa cancer cell lines, respectively (Table 3). They also yielded low inhibition at high concentrations against Vero cells (CC_{50} > 800 µg/ml and 663 µg/ml, respectively). The acetone extract from *L. guatemalensis*, and to some extent the acetone extract from *P. americana*, showed moderate activity against the breast and HeLa lines (IC_{50} = 226 µg/ml and 387 µg/ml, respectively), and low inhibition at high concentrations against the Vero line (CC_{50} > 800 µg/ml). The other species showed high IC_{50} and/or low

Table 2. The effect of acetone and methanol extracts on cancer cell lines.

Plant species	Percent inhibition (200 µg/ml)*									
	Breast		HeLa		Skin		Tongue		Vero	
	A	M	A	M	A	M	A	M	A	M
Acacia farnesiana	0	0	0	39±4	0	0	19±2	3±1	0	0
Acalypha guatemalensis	0	0	0	0	0	0	10±5	14±7	5±1	0
Achillea millifolium	0	0	0	0	0	0	0	5±2	-	-
Allium sativum	0	0	0	0	0	0	0	0	-	-
Anethum graveolens	5±3	0	0	4±2	0	0	0	0	-	-
Anthemis oppositifolia	0	0	-	-	8±4	6±1	0	0	0	0
Arnica montana	37±1	0	0	0	0	0	0	0	0	-
Asclepias curassavica	0	0	0	0	0	0	4±2	6±3	-	-
Baccharis trinervis	0	0	0	0	0	0	0	0	-	-
Bourreria huanita	0	0	0	0	0	0	0	0	0	0
Brosimum alicastrum	0	0	0	0	0	0	0	0	-	-
Brugmansia candida	0	0	0	0	0	0	0	0	-	-
Bursera simaruba	6±2	96±2	0	0	0	29±9	0	0	-	0
Casimiroa edulis	0	0	0	9±6	0	0	0	0	-	-
Cedrela odorata	0	34±6	0	75±4	0	0	0	0	-	0
Cinnamomum zeylanicum	21±7	0	0	0	0	0	0	0	-	-
Citrus sinensis	0	0	0	0	0	0	0	0	-	-
Coffea arabica	0	0	0	0	0	0	7±3	0	-	-
Costus pictus	0	0	0	27±11	0	0	0	0	-	-
Crotalaria longirostrata	23±1	0	41±5	0	62±12	0	61±8	0	49±2	-
Cupressus lusitanica	0	0	0	0	0	0	0	4±2	0	3±2
Equisetum arvense	6±1	0	15	0	0	0	0	0	0	0
Eriobotrya japonica	0	0	34±6	32±7	0	0	0	0	0	0
Euphorbia lancifolia	0	0	0	0	0	0	0	0	0	0
Fleischmannia pycnocephala	0	0	0	1	5±2	4±2	0	0	0	0
Hibiscus sabdariffa	0	0	0	0	0	0	0	0	0	-
Ixora coccinea	0	0	0	9±3	0	0	0	0	-	-
Jatropha curcas	0	0	0	0	0	0	0	0	0	0
Lantana camara	0	0	0	0	24±5	20±11	0	0	0	-
Lippia dulcis	0	0	0	0	0	0	2±1	0	-	-
Lippia graveolens	0	0	5±1	0	0	0	0	10±3	0	0
Litsea guatemalensis	70±6	60±1	0	11±2	0	0	0	0	0	-
Mirabilis jalapa	4±1	0	0	0	27±3	0	5±1	0	0	0
Murraya paniculata	0	0	7±1	0	0	0	0	0	0	0
Nicotiana tabacum	0	0	0	0	6±1	0	12±4	0	0	0
Ocimum basilicum	3±1	0	36±8	5±2	0	0	0	0	28±2	-
Ocimum micranthum	0	0	0	8±5	0	7±1	0	0	0	0
Origanum vulgare	0	0	0	0	0	0	14±9	0	0	-
Passiflora lingularis	0	0	32±7	15±6	12±3	5±2	6±3	0	0	0
Persea americana	97±1	9±1	94±1	49±1	15±2	0	0	0	4±1	0
Petiveria alliacea	0	0	0	0	7±2	0	0	0	3±1	0
Pimenta dioica	0	0	0	0	19±4	3±1	6±1	0	0	0
Piper auritum	0	0	0	0	0	0	0	0	-	-
Pithecellobium dulce	73±7	34±1	36±1	22±5	0	0	24±1	0	54±3	4±2
Priva lappulacea	46±4	0	0	0	0	0	0	0	0	-
Prunus persica	0	0	8±2	0	0	0	0	3±1	0	0
Punica granatum	0	0	21±5	0	3±2	0	0	0	7±3	0
Rauvolfia tetraphylla	0	0	0	20±1	0	0	0	0	-	-
Rhus terebinthifolia	0	0	0	0	0	0	0	0	6±1	0

Table 2 cont'd

Rosmarinus officinalis	2±1	3±1	0	0	19±6	0	0	0	-	-
Rubus villosus	0	0	0	9±4	0	0	0	0	5±1	-
Senna occidentalis	0	0	0	0	11±7	0	6±3	0	-	-
Solanum torvum	9±3	0	0	0	0	0	0	0	0	-
Solanum umbellatum	0	0	68±3	69±1	4±1	0	0	0	18±4	0
Spondias purpurea	18±1	0	0	0	0	0	0	0	-	-
Stigmaphyllon ellipticum	0	0	0	0	0	0	0	5±3	0	0
Tagetes filifolia	0	0	12±1	0	0	0	0	0	-	-
Tagetes lucida	0	0	0	0	0	3±1	0	0	5±1	0
Taraxacum officinale	0	0	0	0	0	0	6±3	0	-	-
Thevetia peruviana	30±4	34±10	60±10	68±1	51±5	62±7	39±7	42±12	62±4	59±2
Thymus vulgaris	0	0	6±3	0	0	0	0	4±1	-	-
Tridax procumbens	0	0	0	0	0	0	0	0	-	-
Vetiveria zizanioides	0	0	0	0	0	0	0	0	0	11±5

*All comparisons between values at 60% or greater inhibition and their controls were significantly different at $P \leq 0.001$.

Table 3. Half-maximum inhibitory concentration (IC_{50}) for cancer lines and half-maximum cytotoxicity concentration (CC_{50}) for the Vero cell line.

Cancer cell line/plant species	IC_{50} (µ/ml)		CC_{50} (µ/ml)	
	A	M	A	M
Breast				
Thevetia peruviana	487	592	663	<6
Bursera simaruba	-	75	-	>800
Pithecellobium dulce	734	-	267	-
Ocimum micranthum	>800	-	>800	-
Litsea guatemalensis	226	-	>800	-
HeLa				
Thevetia peruviana	30	85	663	<6
Persea americana	387	667	>800	>800
Solanum umbellatum	365	315	278	354
Skin				
Thevetia peruviana	800	25	663	<6
Crotalaria longirostrata	168	-	136	-
Tongue				
Thevetia peruviana	>800	>800	663	<6
Crotalaria longirostrata	492	-	136	-

CC_{50} values.

Microbial inhibition

Thirteen (21.3%) of the 61 species tested showed growth inhibition at 60% or greater against one or more microbes (Table 4). Acetone extracts from *Eriobotrya japonica* (Thumb.) Lindl. (Rosaceae), *Mirabilis jalapa* L. (Nyctaginaceae), *P. americana*, *Pimenta dioica* (L.) Merr. (Myrtaceae), *Priva lappulacea* (L.) Pers. (Verbenaceae), and *Rubus villosus* Lasch. (Rosaceae) were active against *S. aureus*. Methanol extracts from *B. simaruba*, *C. odorata*, and *Murraya paniculata* (L.) Jack (Myrtaceae) were also active against *S. aureus*, as were the acetone and methanol extracts from *P. dulce* (Table 4). Methanol

Table 4. The effect of acetone and methanol extracts on microbes.

Genus/Species	% Inhibition (1000 µg/ml)*									
	S. aureus		*S. mutans*		*E. coli*		*L. acidophilus*		*C. albicans*	
	A	M	A	M	A	M	A	M	A	M
Acacia farnesiana	5±2	38±2	8±4	57±1	16±6	32±3	0	0	0	0
Acalypha guatemalensis	22±1	0	0	0	10±5	0	0	0	0	0
Achillea millefolium	0	0	9±4	51±5	8±1	95±1	20±5	98±1	0	8±3
Allium sativum	0	0	0	24±1	4±1	0	9±5	0	0	0
Anethum graveolens	5±2	0	0	7±3	0	10±4	-	-	0	0
Arnica montana	22±2	0	0	26±1	0	19±3	0	21±2	0	0
Asclepias curassavica	0	16±5	12±3	19±3	0	11±1	35±5	0	0	27±2
Baccharis trinervis	0	33±4	0	0	12±2	0	0	0	5±1	0
Bourreria huanita	0	0	0	0	0	0	0	0	0	0
Brosimum alicastrum	0	12±3	10±4	0	6±2	0	0	0	0	0
Brugmansia candida	0	0	11±3	4±1	38±1	0	0	16±7	0	0
Bursera simaruba	0	68±1	23±2	36±1	14±3	56±2	0	0	0	0
Casimiroa edulis	0	58±1	12±2	37±1	12±1	5±1	0	27±6	0	0
Cedrela odorata	33±4	84±3	0	0	0	-	-	-	0	0
Cinnamomum zeylanicum	0	0	0	52±1	0	40±2	-	-	0	0
Citrus sinensis	22±4	35±9	-	0	0	0	12±5	24±5	-	0
Coffea arabica	0	0	0	0	29±5	9±4	22±1	19±2	0	0
Costus pictus	0	0	0	0	13±7	0	17±1	39±4	0	0
Crotolaria longirostrata	0	0	0	0	22±1	0	65±3	30±1	16±4	10±7
Cupressus lusitanica	0	0	0	0	0	0	0	0	7±1	0
Equisetum arvense	0	0	0	0	0	0	0	0	0	0
Eriobotrya japonica	62±3	15±5	-	31±4	89±1	15±6	0	0	0	0
Euphorbia lancifolia	0	0	0	0	0	0	0	0	0	0
Fleischmanni pycnocephala	0	0	0	0	0	0	0	0	0	0
Hibiscus sabdariffa	0	0	15±7	19±3	21±2	0	27±2	29±1	0	21±1
Ixora coccinea	0	0	0	0	0	0	20±8	0	0	0
Jatropha curcas	0	0	20±5	16±1	10±6	0	0	0	0	0
Lantana camara	0	0	0	0	18±5	0	0	6±2	0	0
Lippia dulcis	0	0	14±2	15±1	0	0	22±2	21±4	0	20±7
Lippia graveolens	0	0	0	0	0	0	18±1	0	0	0
Litsea guatemalensis	0	0	41±5	17±4	0	0	-	-	0	0
Mirabilis jalapa	60±6	48±5	36±3	0	17±4	0	0	0	0	9±3
Murraya paniculata	0	98±1	9±3	0	15±3	0	0	0	0	0
Nicotiana tabacum	0	0	0	0	0	0	0	0	0	0
Ocimum basillicum	0	0	15±3	12±3	16±6	26±1	34±2	10	0	0
Ocimum micranthum	0	0	32±3	0	0	0	0	0	0	0
Origanum vulgare	0	0	18±4	1	0	0	0	0	0	29±3

Table 4. Cont'd.

Passiflora lingularis	0	0	0	0	0	0	0	0	0	0
Persea americana	64±5	29±4	41±3	26±2	0	0	15±3	0	0	0
Petiveria alliacea	0	0	0	0	0	0	0	6	0	0
Pimenta dioica	60±3	19±9	18±6	0	43±1	29±5	0	0	0	0
Piper auritum	0	7±1	23±4	33±6	23±2	0	0	0	0	0
Pithecellobium dulce	90±3	85±4	0	61±7	90±1	89±2	-	-	0	0
Priva lappulacea	83±1	0	28±9	13±2	0	0	59±1	0	0	0
Prunus persica	0	0	-	0	14±1	12±3	10±2	15±1	0	0
Punica granatum	44±3	29±7	28±2	23±6	12±4	0	0	0	0	0
Rauvolfia tetraphylla	0	0	0	0	0	0	0	0	0	0
Rhus terebinthifolia	28±9	17±2	24±7	22±6	36±2	18±3	0	0	0	0
Rosmarinus officinalis	23±2	0	0	0	0	17±6	0	0	-	0
Rubus villosus	78±2	16±1	0	0	45±6	38±1	0	0	0	0
Senna occidentalis	0	0	0	18±2	0	0	0	23±6	0	0
Solanum torvum	0	0	0	31±3	0	0	0	0	0	0
Spondias purpurea	0	45±4	-	98±3**	0	0	35±5	13±4	0	0
Stigmaphyllon ellipticum	0	0	39±1	20±7	0	0	18±2	0	0	0
Tagetes filifolia	0	25±1	0	0	0	0	0	0	0	0
Tagetes lucida	0	0	0	0	0	0	17±3	0	0	0
Taraxacum officinale	28±4	0	0	0	0	0	0	19±5	0	0
Thevetia peruviana	0	0	16±5	0	24±2	0	0	0	0	0
Thymus vulgaris	0	0	0	13±3	0	0	22±3	25±7	0	31±6
Tridax procumbens	0	0	0	0	0	0	0	0	0	0
Vetiveria zizanioides	0	0	0	0	0	0	0	0	0	0

*All comparisons between values at 60% or greater inhibition and their controls were significantly different at $P \leq 0.001$ except for S. purpurea** which was significantly different at $P \leq 0.03$.

extracts from *P*. *dulce* and *Spondias purpurea* L. (Anacardiaceae) were inhibitory to the growth of *S. mutans*; no acetone extract was active against *S. mutans* (Table 4). The acetone extract from *E*. *japonica*, the methanol extract from *Achillea millefolium* L. (Asteraceae), and the acetone and methanol extracts from *P*. *dulce* were active against *E*. *coli*. The methanol extract of *A*. *millefolium* and the acetone extract of *C. longirostrata* were the only extracts active against *L*. *acidophilus*. No extracts were active against *C. albicans* (Table 4).

Minimum inhibitory concentrations (MICs)

The acetone extracts of *M. jalapa*, *P*. *dioica*, and *R. villosus* yielded MIC values of 250 µg/ml against *S. aureus* (Table 5). The methanol extract of *B. simaruba* produced an MIC of >1000 µg/ml against *S. aureus*, and a MIC of 500 µg/ml against *E. coli* (Table 5) even though it was not inhibitory to *E. coli* in the inhibition assay (Table 4). Extracts from *E*. *japonica* and *P*. *dulce* yielded extracts with a MIC of 1000 µg/ml; all other extracts yielded MIC values >1000 µg/ml and were not considered inhibitory.

DISCUSSION

Our study along with Kufer et al. (2005) and Comerford (1996) note a wide variety of uses for

Table 5. Minimum inhibitory concentrations (MIC) for Guatemalan medicinal plants that showed greater than 60% inhibition against microbes.

Plant species (Extract)*	MIC (µg/ml)	
	S. aureus	***E. coli***
Achillea millefolium (M)	-	>1000
Bursera simaruba (M)	>1000	500
Cedrela odorata (M)	>1000	
Eribotrya japonica (A)	>1000	1000
Lantana camara (M)	>1000	-
Priva lappulacea (A)	>1000	
Mirabalis jalapa (A)	250	-
Murraya paniculata (A)	>1000	>1000
Persea americana (A)	>1000	-
Pimenta dioica (A)	250	-
Pithecellobium dulce (A,M)	>1000	1000
Rubus villosus (A)	250	-
Spondias purpurea (A)	>1000	-

*A=acetone extract; M=methanol extract; blank space indicates no inhibition per Table 4.

the medicinal plants selected for this study (Table 1). This suggests that these resources are valuable to rural Guatemalans and need to be conserved. Overall, 16 (25.4%) of 63 species were inhibitory to one or more cancer cell lines and/or one or more microbes at the 60% or greater level. Eight species were inhibitory to one or more cancer cell lines and eight were inhibitory to one or more microbes (Tables 2 and 4). Of those active against cancer cells, extracts from *B*. *simaruba* and *L*. *guatemalensis* demonstrated significant inhibition at low concentrations (IC_{50} 75 and 226 µg/ml, respectively) against the breast cell line and showed low inhibition at high concentrations (CC_{50} >800 µg/ml) against the non-cancerous Vero cells (Table 3). The acetone extract from *T*. *peruviana* also demonstrated significant activity against the HeLa cell line (IC_{50} 30 µg/ml vs CC_{50} 663 µg/ml). *P*. *americana* showed some activity against the HeLa line and with further fractionation this species might prove effective against this line. For the eight species that were active against one or more microbes three (*M*. *jalapa*, *P*. *dioica* and *R*. *villosus*) registered a MIC of 250 µg/ml against *S*. *aureus*. *B*. *simaruba* was inhibitory to *S*. *auerus* (Table 4) but the MIC for the methanol extract was >1000 µg/ml (Table 5). Interestingly the methanol extract from *B*. *simaruba* was almost significant at 54% inhibition to *E*. *coli* (Table 4) and that level of inhibition was reflected in a moderately inhibitory MIC of 500 µg/ml against *E*. *coli* (Table 5). Extracts from *C*. *odorata*, *C*. *longirostrata*, *B*. *simaruba*, *P*. *americana*, and *P*. *dulce* were inhibitory to both cancer cell lines and microbes (Table 2 and 4). However, extracts from these five species did not demonstrate significant IC_{50}, CC_{50}, or MIC values (Tables 3 and 5). The stated uses of these species by villagers did not include cancer and microbial diseases (Table 1) so likely the ethnomedical use will not change. Even so, because these species were active against cancer cells and microbes further study of these species may yield promising results.

One focus was to identify medicinal plant species that might be used to improve oral hygiene. Specific emphasis was on plant species demonstrating activity against *S. mutans* and *L. acidophilus* both of which may contribute to cavity formation, and those active against the tongue cancer cell line. *S. purpurea* and *P. dulce* demonstrated significant inhibitory activity against *S. mutans* (Table 4). *C. longirostrata* was inhibitory to the tongue cancer cell line (Table 2), and this species along with *A. millefolium (*and *P. lappulacea* was almost inhibitory at 59% inhibition) were active against *L. acidophilus.* These species merit further investigation as to their efficacy to prevent or treat diseases of the oral cavity.

Several species reported in this study have been reported elsewhere to have activity against human diseases. For example, Johnson (1999) refers to extracts from *B. simarubra* and *P. americana* as being used to treat stomach cancer and tumors, respectively, and in our study these species were active against breast and cervical cancer cells, respectively. Additionally, *S. umbellatum* is an important medicinal plant in some cultures but was not reported to have activity against cancer cell lines (Johnson, 1999).

However, in our study this species was active against cervical cancer cells. In summary, data from this study yielded 11 significantly active species and Cates et al. (2013) noted seven additional active species. Miller

(2014) found 11 other Guatemalan species that produced essential oils which were highly active against the same set of microbes used in this study which brings the total to 29 active medicinal plant species. Future work is needed to determine the pharmacological activity and cytotoxicity of active components. For example, *T. peruviana* was active against the HeLa cell line but is well known for its cytotoxicity (Bandara et al., 2010). Additional studies of the active species might include characterizing the active compounds, and *in vitro* and *in vivo* investigations of their cytotoxicity, mechanism(s) of action, and ultimately their efficacy in preventing and treating diseases.

Conclusion

Sixteen species of medicinal plants were found to be inhibitory to one or more cancer cell lines and/or microbes. Based on cytotoxicity to the Vero cell line, high IC_{50} values and low CC_{50} values, and high MIC values several of these species may not merit further study. However, seven species (*B. simaruba*, *E. japonica, L. guatemalensis*, *M. jalapa*, *P. dioica*, *R. villosus*, *T. peruviana*) merit additional investigation based on their inhibition, IC_{50}/CC_{50} values, and MIC values. With regard to oral hygiene four species (*A. millefolium*, *C. longirostrata*, *P. dulce*, *S. purpurea*) merit further fractionation and testing against various oral diseases.

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Conflict of interests

The author(s) have not declared any conflict of interests.

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