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Evaluation of essential oils from 22 Guatemalan medicinal plants for *in vitro* activity against cancer and established cell lines

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Plant species which produce essential oils are important in the healthcare of rural Guatemalans. Steam distilled essential oils from 22 medicinal plant species were analyzed for activity against tongue, skin, and stomach cancer cell lines using a neutral red assay, Vero C1008 cells to assess cytotoxicity, and [^{3}H]-thymidine incorporation assay to assess inhibition of cancer cell proliferation. IC_{50}, CC_{50}, and therapeutic indices were determined. IC_{50} values indicated that all oils showed inhibitory activity against one or more cancer cell lines. Highly inhibitory IC_{50} values (0.10 µL/mL or less) indicated that *Citrus aurantiifolia* (Christm.) Swingle (Rutaceae) oil was significantly inhibitory to all three cancer cell lines, *Origanum vulgare* L. (Lamiaceae) and *Teloxys ambrosioides* (L.) W. A. Weber (Chenopodiaceae) oils were highly inhibitory to two cell lines, and *Lippia graveolens* Kunth (Verbenaceae) oil was highly inhibitory to one cell line. TI values equal to or greater than one showed significantly higher cytotoxicity to cancer cells compared to the Vero cell line for *Ruta chalepensis* L. (Rutaceae), *Citrus limetta* Risso (Rutaceae), *C. aurantium* L. (Rutaceae), *Rosmarinus officinalis* L. (Lamiaceae), and *O. vulgare*. Essential oils from *L. graveolens*, *O. vulgare*, and *T. ambrosioides* yielded high percentages (>96%) of decreased cell proliferation at low oil concentration (0.05 µL/mL). Results indicate that essential oils were more toxic to cancer cells than to cells from an established cell line, and such oils can be highly suppressive to DNA synthesis and cancer cell growth.

Key words: Essential oils, medicinal plants, IC_{50}, anticancer activity, Guatemala.

INTRODUCTION

The use of medicinal plants is important to the health care of Guatemalans (Hautecoceur et al., 2007; Cates et al., 2013) and there is a need to determine the efficacy of these plants against human diseases (Comerford, 1996; Kufer et al., 2005; Adams and Hawkins, 2007). For example, essential oils from 11 species collected in Guatemala yielded high levels of inhibition and low MIC values against pathogenic microbes (Miller et al., 2015).

In the study reported here, the activity of essential oils from 22 species against tongue, skin, and stomach cancer cell lines and a non-cancerous Vero line is reported. A neutral red assay was used to determine the cytotoxicity of each oil using IC_{50} (half the maximal inhibitory concentration) and CC_{50} (cytotoxic concentration to cause death of 50% of viable cells) values, a therapeutic index was then calculated for each oil, and the degree of inhibition of cancer cell proliferation using the [^{3}H]-thymidine incorporation assay was determined.

Essential oils are complex mixtures of monoterpenes, sesquiterpenes and phenolics (Carson and Riley, 1995; Radulescu et al., 2004), and are known to have biological activity against cancer cell lines (Edris, 2007; Bhalla et al., 2013; Gautam et al., 2014; Raut and Karauppayil, 2014). Oil activity has been shown to be a sum of the effects of individual components based on the ratio of the different constituents and not necessarily on the quantity of one component (Kalemba and Kunicka, 2003; Houghton et al., 2007).

Essential oils show a high degree of quantitative and qualitative variability among species, within a species, and among tissue types due to genetic factors, seasonality, and environmental factors (Valladares et al., 2002; Lahlou, 2004; Bakkali et al., 2008; Barra, 2009; Padialia et al., 2014; Grulova et al., 2015; Salmasi et al., 2016). This variation indicates strong potential for synergistic interactions among oil components (Biavatti, 2009; Rosso et al., 2015) and specific mechanisms of action toward a particular cancer cell line or disease organism (Wittstock and Gershenzon, 2002; Rajesh and Howard, 2003; Savelev et al., 2003; Salminen et al., 2008). These characteristics suggest that the development of essential oils might be useful in anticancer treatments and cancer therapies (Patel and Gogna, 2015).

MATERIALS AND METHODS

Plant selection and tissue collection

Plants species were collected in Guatemala from 2007 to 2009 in the villages of Tuticopote, Salitrón, Roblarcito, Olopa, and San Juan Ermita in the Chiquimula Department. Additional collections were made at the Museo Odontológico de Guatemala and the Jardín Botánico Maya, Guatemala City and the Coleccion y Huerto Productivo de Plantas Medicinales, Centro Experimental y Docente de Agronomia, Guatemala City. Vouchers with detailed collection information were deposited in the Herbaria at the CUNORI Campus, University of San Carlos, Chiquimula, Guatemala and at Brigham Young University (BRY) herbarium, Provo, UT USA.

For each sample about 300 g of plant tissue was bagged, labelled, placed on dry ice, and stored in a -80°C ultralow (Isotemp Basic, Thermo Electron Corporation, Asheville, NC USA) at BYU.

Essential oil extraction and preparation

Essential oils were extracted using a steam distillation apparatus (Scientific-Glass, Rancho Santa Fe, CA, USA) following Luque de Castro (1999) and Charles and Simon (1990). In order to obtain sufficient oil for the assays, multiple samples of each species were extracted for approximately 4 h followed by oil removal from the receiver of the apparatus by pipette.

To aid in the separation of oils from the water and glass surfaces, 125 µL of diethyl-ether (Mallinckrodt-Baker, Phillipsburg, NJ, USA) was added to the receiver. The oil/diethyl-ether mixture was removed, placed in vials and dehydrated using anhydrous sodium sulfate (EMD Chemicals, Darmstadt, Germany). Oils were separated from sodium sulfate by adding an additional 200 µL of diethyl-ether, and then the diethyl-ether was evaporated from the oil/diethyl-ether mixture using pressurized nitrogen (approximately 35 s). The resulting purified essential oil was then placed in an amber vial, weighed, and stored at -80°C until analyzed (Miller et al., 2015). Oil yields from multiple extractions of tissue from each plant species were averaged and expressed as % yield (w/w) (Table 1).

Cell lines and cytotoxicity

Cancer cell lines chosen for bioactivity testing were stomach (ATCC CRL-1739, human epithelial gastric adenocarcinoma; ATCC, Manassas, VA, USA), skin (ATCC CRL-1619, human epithelial malignant melanoma; ATCC), and tongue (ATCC CRL-2095, human epithelial squamos carcinoma; ATCC). The established Vero C 1008 line (Monkey Kidney cells, ATCC CRL-1586, epithelial kidney normal; ATCC) was chosen to determine cytotoxicity of the essential oils and for calculating therapeutic indices.

Skin, tongue, and Vero C 1008 cell lines were grown in DMEM (GIBCO, Grand Island, NY, USA) fortified with 10% Fetal Bovine Serum (FBS) (ATCC), 5 mL of 1M HEPES (Hyclone, Logan, UT, USA), 2.5 mL of 100 mM sodium pyruvate (Hyclone), and 5 mL of 10 mg/mL gentamycin (Sigma-Aldrich, St. Louis, MO, USA). Stomach cells were grown in Ham's F-12 Kaighn's Modification media (Hyclone) fortified with FBS (10%), 5 mL of 1M HEPES, and 5 mL of 10 mg/mL gentamycin.

Method adaptation

Assessment of the bioactivity of essential oils can be problematic due to the highly volatile nature of the oils and their lack of solubility (Donaldson et al., 2005). Volatile components were found to cross-contaminate adjacent wells of 96-well plates even at low concentrations, thereby leading to inaccurate estimations of minimum inhibitory concentration (MIC) and IC_{50} values. Donaldson et al. (2005) proposed the addition of 2% biological grade agar (w/v) to the culture media to remedy this problem in microbial tube dilution assays. In order to adapt this method to allow the use of 96-well plates, 15% biological grade agar (v/v) was added to the cell culture media. The addition of 15% agar (v/v) mixed with cell culture media consistently showed no inhibitory

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effects on the growth of untreated cells in preliminary trials. The resulting mixture of inert agar also maintained a stable emulsion over a 24 h period and minimized oil volatility.

Cell culture techniques

DMEM agar-media was prepared by adding melted molecular biology grade agar (Fisher, Fair Lawn, NJ, USA) to media at 15% v/v ratio at room temperature and then allowing the mixture to cool. FBS (10%) was then added followed by 5 mL of 1M HEPES, 2.5 mL of 100 mM sodium pyruvate, and 5 mL of 10 mg/mL gentamycin. Ham's F-12 Kaighn's Modification agar-media was prepared in the same manner with the omission of sodium pyruvate. All cell lines were grown to 90% confluency in 175cm^2 flasks (Sarstedt) at 37°C and 5% CO_2 and then seeded in 96-well plates. Stomach cells were seeded at a density of 7.0 x 10^4, skin cells at 6.0 x 10^4, tongue cells at 5.0 x 10^4, and Vero C 1008 cells at 2.0 x 10^4. Each well was filled with 150 µL of complete media and then placed in an incubator at 37°C and 5% CO_2.

Seeded plates were removed from the incubator after 24 h and the media was also removed. Two essential oils and their controls were analyzed on each plate and each concentration was replicated three times. Essential oils were serially diluted in agar media resulting in final concentrations of 7.0, 3.5, 1.75, 0.88, 0.44, 0.22, 0.11 and 0.05 µL/mL. 200 µL of the appropriate essential oil concentration was then added to each well. Controls consisted of 200 µL of agar media in wells with no additives. All edge wells were filled with 200 µL of sterilized double distilled water (DDH_2O) to control edge effects. Each plate was returned to the incubator for an additional 24 h

Neutral red assay to determine IC_{50} and CC_{50} values

The neutral red (NR) assay was chosen for determining IC_{50} and CC_{50} because of its accuracy in the quantitative assessment of *in vitro* cytotoxicity (Borenfreund and Puerner, 1985; Babich and Borenfreund, 1991; Schröterová et al., 2009). Plates were removed from the incubator after 24 h and phosphate buffered saline was used to gently wash and remove all traces of the essential oil and the agar-media from the wells. NR dye solution was made using 0.33 mg/mL NR solution (3-aminom-dimethylamino-2-methyl-phenazine hydrochloride in DBPS; Sigma-Aldrich) and then added to make a 10% NR media mixture.

This solution was added to each well excluding edge wells which were filled with sterilized DDH_2O. Plates were then incubated for 3 h after which the NR media mixture was removed and discarded. A fixative solution (1% $CaCl_2$ in 0.5% formaldehyde; Mallinckrodt, Phillipsburg, NJ, USA) was added, removed after 30 s of exposure, and then a solubilization solution was added (1% acetic acid; EM Science, Gibbstown, NJ, USA, in 50% ethanol; Decon Labs, King of Prussia, PA, USA). Cell viability was measured using a Fusion α-HT Universal Microplate Analyzer (Packard Instruments, Meriden, CT, USA) with 540 nm filter and 690 nm reference filter.

Final values were generated by subtracting the 690 value from the 540 value followed by correction of the data by subtracting the average value generated from the blank edge wells. The values of the three replicate trials were first averaged and then used to create a dose-response curve from which final IC_{50} and CC_{50} values were determined. A Therapeutic Index was calculated using the ratio CC_{50}/ IC_{50} (Greer et al., 2010).

Determination of cell proliferation

Oils form this assay were selected based on the lowest IC_{50} values within a cancer cell line and the consideration of the IC_{50} values among cancer cell lines within a plant species (Table 4). The [^{3}H]-thymidine incorporation assay is a measure of cell proliferation levels based on the synthesis of new DNA (Sugihara et al., 1992; Marimpietri et al., 2007; Zhang et al., 2008), and consequently, a high value which indicates the percent decrease in cell proliferation (Table 4).

Cancer cells were seeded into 96-well plates at previously noted densities, incubated (37°C, 5% CO_2) for 4 h, and then oils were added at a concentration that yielded the IC_{50} value for that oil. This mixture was then incubated for 24 h. The oil/media mixture was removed and the cells were washed once with fresh media followed by the addition of 200 ml of fresh media with thymidine (Amersham, Piscataway, NJ, USA).

0.1 µL thymidine (185 GBq/mmol) was used for skin and tongue lines and 0.15 µL for stomach line. Plates were incubated for 4 h and then harvested using a multi-well harvester (Inotech Biosystems International, Rockville, MD, USA) with collection onto glass fiber filters. Each filter was placed into approximately 2.5 mL of scintillation fluid (MP Biomedicals, Solon, OH, USA) and results of radioactivity were measured in cpm on a scintillation counter (Beckman Coulter, Brea, CA, USA). Those values were used to calculate a percent decrease in cell proliferation relative to controls.

Statistical analysis

For cell proliferation assay, four replicates of each oil along with their controls were assayed on each plate. Statistical significance was measured using the Student's t-test comparing the cpm values of the essential oil treatment for each species relative to the controls ($P \leq 0.05$).

RESULTS

Essential oil yield

13 species yielded 0.25% (w/w) or less essential oil, 7 species between 0.25 to 0.5%, and 2 species greater than 0.5% yield (Table 1). The families Asteraceae, Lamiaceae, Myrtaceae, Lauraceae, and Rutaceae were represented by multiple species. Species in the Lamiaceae averaged 0.43% (w/w) essential oil, Asteraceae and Lauraceae 0.35%, Myrtaceae 0.27%, and Rutaceae 0.14% (Table 1). *Origanum vulgare* L. (Lamiaceae) produced the largest amount of essential oil with a leaf content of 0.66% (Table 1).

IC_{50} and CC_{50}

All oils assayed showed inhibitory activity against one or more cancer cell lines (Table 2). Highly inhibitory IC_{50} values of 0.10 µL/mL or less were observed against cancer cell lines in eight instances from four species (12% of total recorded IC_{50} values). Additionally, 28 moderately inhibitory IC_{50} values (between 0.10 and 0.30 µL/mL) were observed for 15 species (42% of total recorded IC_{50} values), resulting in a total of 36 instances of an IC_{50} of 0.30 µL/mL or less. Overall, 10 IC_{50} values (45%) for skin cell line, 12 (54%) for stomach cell line, and 14 values (64%) for tongue cell line were below 0.30

Table 1. Species, family, common name, tissue type and percent yield per species for Guatemalan medicinal plants extracted by steam distillation distillation.

Species	Family	Common name	Tissue	% Yield (w/w)
Achillea millefolium L.	Asteraceae	milenrama	Aerial Portion	0.11
Anethum graveolens L.	Apiaceae	hinojo	Aerial Portion	0.07
Bixa orellana L.	Bixaceae	achiote	Seed	0.12
Buddleja americana L.	Buddlejaceae	salvia santa	Leaf	0.09
Cinnamomum zeylanicum Blume	Lauraceae	canela	Leaf	0.45
Citrus aurantiifolia (Christm.) Swingle	Rutaceae	limón criollo	Leaf	0.25
C. aurantium L.	Rutaceae	naranja	Leaf	0.08
C. limetta Risso	Rutaceae	lima	Leaf	0.17
Cupressus lusitanica Mill.	Cupressaceae	ciprés	Leaf	0.93
Eucalyptus globulus Labill.	Myrtaceae	eucalipto	Leaf	0.35
Lippia graveolens Kunth	Verbenaceae	oregano	Leaf	0.45
Litsea guatemalensis Mez	Lauraceae	laurel	Leaf	0.24
Mentha piperita L.	Lamiaceae	menthol piperita	Aerial Portion	0.50
Ocimum basilicum L.	Lamiaceae	albahaca	Aerial Portion	0.33
Origanum vulgare L.	Lamiaceae	oregano de castillo	Aerial Portion	0.66
Pinus oocarpa Schiede ex Schltdl.	Pinaceae	pino	Leaf	0.04
Piper auritum Kunth	Piperaceae	santa maria	Leaf	0.27
Psidium guajava L.	Myrtaceae	guayabo	Leaf	0.19
Rosmarinus officinalis L.	Lamiaceae	romero	Leaf	0.23
Ruta chalepensis L.	Rutaceae	ruda	Aerial Portion	0.07
Tagetes filifolia Lag.	Asteraceae	anís de monte	Aerial Portion	0.50
Teloxys ambrosioides (L.) W.A.Weber	Chenopodiaceae	apasote	Aerial Portion	0.12

µL/mL. Calculation of the average IC_{50} for each cell line shows the tongue cell line, having the highest inhibitory average IC_{50} with 0.29 µL/mL, followed by the stomach cell line at 0.32 µL/mL and skin cell line at 0.49 µL/mL.

Highly inhibitory IC_{50} values of 0.10 µL/mL or less were produced by oils from *Citrus aurantiifolia* (Christm.) Swingle (Rutaceae) (against all 3 cancer cell lines), *Origanum vulgare* L. (Lamiaceae) (2 cell lines), *Teloxys ambrosioides* (L.) W.A. Weber (Chenopodiaceae) (2 cell lines), and *Lippia graveolens* Kunth (Verbenaceae) (1 cell line). All values from *C. aurantiifolia* were < 0.05 µL/mL (0.05 µL/mL was the smallest measurable IC_{50} value in this assay). Oils from *C. aurantiifolia, Teloxys ambrosioides, L. graveolens*, and *O. vulgare* were most inhibitory to the tongue cell line (all equal to or less than 0.10 µL/mL).

The skin cell line was most inhibited by *C. aurantiifolia, T. ambrosioides, O. vulgare, L. graveolens* and *Cinnamomum zeylanicum* Blume (Lauraceae) (all equal to or less than 0.18 µL/mL), and stomach cells were most inhibited by *C. aurantiifolia*, *Citrus aurantium* L. (Rutaceae), *Citrus limetta* Risso (Rutaceae), *L. graveolens, Litsea guatemalensis* Mez (Lauraceae), *O. vulgare*, *Pinus oocarpa*, *Psidium guajava* L. (Myrtaceae), and *Eucalyptus globulus* Labill. (Myrtaceae) (IC_{50} values were equal to or less than 0.19 µL/mL).

All essential oils were shown to be cytotoxic to Vero C 1008 cell line at some concentration (Table 2). 10 oils (45%) produced highly cytotoxic CC_{50} values of 0.10 µL/mL or less, and 9 oils (41%) produced moderately inhibitory CC_{50} values (between and 0.30 µL/mL). In total 19 oils (86%) produced a CC_{50} value below 0.30 µL/mL against the Vero cells. The most cytotoxic CC_{50} values were produced by oils from *Bixia orellana* L. (Bixaceae), *C. aurantiifolia*, *Cupressus lusitanica* Mill. (Cupressaceae), *Buddleja americana* L. (Buddlejaceae), and *T. ambrosioides.* Least toxic species were *Ruta chalepensis* L. (Rutaceae) and *Tagetes filifolia* Lag. (Asteraceae) (Table 2).

14 (21%) therapeutic index (TI) values, where TI value was equal to or greater than 1 indicated a significantly higher cytotoxcity to cancer cells compared to the established Vero cell line (Table 3). For *R. chalepensis* all three TI values were over 1, and two values over 1 which were recorded for *C. limetta, C. aurantium, Rosmarinus officinalis* L. (Lamiaceae), and *O. vulgare*, and one value greater than or equal to 1 was recorded for *E. globulus, P. oocarpa,* and *L. graveolens.* Ten TI values were not calculated due to IC_{50} or CC_{50} values being below the smallest measurable value for this

Table 2. IC_{50} values (µL/mL) for cancer cell lines, and CC_{50} (µL/mL) values for the Vero C 1008 line, for essential oils of Guatemalan medicinal plants *in vitro.*

Species	C1008	Cancer Cell Lines		
		Tongue	Skin	Stomach
A. millefolium[#]	0.2	0.28	0.29	0.4
A .graveolens[#]	0.3	0.42	0.64	0.87
B. orellana[#]	<.05*	0.79	0.65	0.29
B. americana[#]	0.06	0.27	0.44	0.39
C. zeylanicum	0.08	0.16	0.18	0.25
C. aurantiifolia	<.05*	<.05*	<.05*	<.05*
C. aurantium[#]	0.25	0.16	0.37	0.17
C. limetta[#]	0.31	0.18	0.62	0.13
C. lusitanica[#]	<.05*	0.22	0.25	0.36
E. globulus	0.17	0.32	0.38	0.16
L. graveolens[#]	0.09	0.07	0.14	0.15
L. guatemalensis[#]	0.11	0.17	0.2	0.19
M. piperita	0.09	0.23	0.4	0.35
O. basilicum	0.14	0.34	0.36	0.39
O. vulgare	0.1	0.08	0.09	0.18
P. oocarpa[#]	0.15	0.15	0.6	0.17
P. auritum[#]	0.17	0.43	0.86	0.41
P. guajava	0.07	0.21	0.28	0.15
R. officinalis	0.26	0.41	0.24	0.21
R. chalepensis[#]	0.72	0.51	0.71	0.64
T. filifolia[#]	0.42	0.7	2.6	>7**
T. ambrosioides[#]	0.06	0.07	0.08	0.66

[#]Oils not previously reported to have been tested on cancer cell lines *in vitro,* *IC_{50} values are below the measurable values of this assay, **IC_{50} values are above the measurable values of this assay.

assay (Table 3).

Cell proliferation

DNA synthesis as measured by cancer cell proliferation was significantly decreased ($P \leq 0.026$) by, exposure to essential oils (Table 4). All oils resulted in a decrease in cell proliferation by at least 50% and 15 of the 22 oils resulted in a decrease of 95% or greater. For skin cell line oils from *T*. *ambrosioides*, *O*. *vulgare* demonstrated high percentages (>97%) of decreased cell proliferation at low oil concentrations of 0.05 µL/mL, respectively.

L. *graveolens* demonstrated a high percentage (>96%) in decreased proliferation for tongue cell line at a concentration of 0.05 µL/mL. None of the oils tested against the stomach cell line showed similar decreases in cell proliferation at low oil concentrations (<10 µL/mL). Average decrease in cell proliferation was greatest for oils effective against the skin cancer cell line (99%), followed by oils against the tongue cell line (88%) and stomach cell line (80%).

DISCUSSION

IC_{50} and CC_{50}

The IC_{50} values for 59% of the oils assayed have not been reported previously (Table 2). All oils assayed showed some inhibitory effect on cancer cells lines (Table 2) and many displayed high inhibition at low concentrations. For *C. aurantium*, *C. limetta*, *E. globulus*, *L. graveolens*, and *O. vulgare*, (and to some extent for *R. officinales* and *R. chalepensis*) the IC_{50} values, therapeutic indices, and cell proliferation decreases are consistent in showing significant inhibition of cancer cells (Tables 2, 3, and 4).

Oil from *C. aurantiifolia* was the most effective oil against all three cancer cell lines with an IC_{50} less than 0.05 µL/mL for each line (Table 2). Oil from *O. vulgare* produced highly inhibitory IC_{50} values against skin and tongue cell lines and *L. graveolens* produced a highly inhibitory IC_{50} value against the tongue cell line. The average IC_{50} value for each of these oils against the three cancer cell lines was 0.12 µL/mL indicating potential for

Table 3. Therapeutic Index values for essential oils from Guatemalan medicinal plants for activity on cancer and established cell lines *in vitro*.

Species	Cancer	Cell	Lines
	Tongue	Skin	Stomach
A. millefolium	0.71	0.69	0.50
A. graveolens	0.71	0.47	0.34
B. orellana	+	+	+
B. americana	0.22	0.14	0.15
C. zeylanicum	0.50	0.44	0.32
C. aurantiifolia	+	+	+
C. aurantium	1.56	0.68	1.47
C. limetta	1.72	0.50	2.38
C. lusitanica	+	+	+
E. globulus	0.53	0.45	1.06
L. graveolens	1.29	0.64	0.60
L. guatemalensis	0.65	0.55	0.58
M. piperita	0.39	0.23	0.26
O. basilicum	0.41	0.39	0.36
O. vulgare	1.25	1.11	0.56
P. oocarpa	1.00	0.25	0.88
P. auritum	0.40	0.20	0.41
P. guajava	0.33	0.25	0.47
R. officinalis	0.63	1.08	1.24
R. chalepensis	1.41	1.01	1.13
T. filifolia	0.60	0.16	+
T. ambrosioides	0.86	0.75	0.09

+Unable to calculate therapeutic index (TI) due to lack of IC_{50} or CC_{50} value.

broad scale cancer cell inhibition. Both oils have been reported to have similar composition (Salgueiro et al., 2003) which may explain their comparable levels of activity and effectiveness (Al-Kalaldeh et al., 2014; Begnini et al., 2014).

Oil from *T. ambrosioides* also produced highly inhibitory IC_{50} values against skin and tongue cell lines (Table 2). Additional oils with IC_{50} values showing moderate inhibition were *C. zeylanicum* (skin), *Litsea guatemalaensis* (skin), and *P. guajava* (stomach) with values equal to or less than 0.21 µL/mL. Additionally, Yuangang et al. (2010) showed that the essential oil from *C*. *zeylanicum* was moderately active against prostate and lung cancer cells and Manosroi et al. (2006) found that the essential oil from *P*. *guajava* was moderately active against human mouth epidermal carcinoma. Oils from both of these plants are known to contain β-caryophyllene oxide, which has been noted for signal cascade inactivation resulting in down-regulation of proliferation and angiogenesis in some cancer cell lines (Park et al., 2011; Kim et al., 2014). Oils from *R. officinalis* also have been reported to have high inhibitory values against a variety of cell lines (Hussain et al., 2010; Wang et al., 2012).

Therapeutic indices further indicated that several oils show potential because TI values greater than 1 indicate reduced cytotoxicty to cells from the established cell line (Table 3). TI values for *C. limetta, C. aurantium, L. graveolens* and *O. vulgare* indicate the potential of these oils against the tongue cell line. Additionally TI values of *C. limetta, C. aurantium* and *E. globulus* indicate potential against the stomach cell line while oils from *O. vulgare* and *R. officinalis* showed similar results against the skin cell line. The oil from *R. chalepensis* was the only oil that generated three TI values greater than 1, although none of the individual IC_{50} values were highly inhibitory.

Additional testing and identification of active components of the oil from this species are needed to determine if similar compounds are active against both non-cancerous and cancerous cells. Average TI values of 2.05 and 1.52 across tongue and stomach cell lines, respectively, were calculated for *C. limetta* and *C. aurantium* (Table 3) possibly indicating broad spectrum activity. *O. vulgare* showed a similar pattern against the

Table 4. The effect of essential oils on per cent decrease in cell proliferation as measured by [^{3}H]-thymidine incorporation (% ± se).

Tongue			Skin			Stomach		
Species	**Oil conc.#**	**% (se) Proliferation decrease**	**Species**	**Oil conc.#**	**% (se) Proliferation decrease**	**Species**	**Oil conc.#**	**% (se) Proliferation decrease**
L. graveolens	0.05	96.6 (0.71)***	*T. ambrosioides*	0.05	97.9 (0.14)*	*C. aurantiifolia*	< 0.05	83.5 (2.57)**
C. aurantium	0.15	74.6 (6.51)**	*O. vulgare*	0.05	99.5 (0.06)*	*C. limetta*	0.10	67.6 (7.52)*
M. piperita	0.20	98.0 (0.23)*	*C. zeylanicum*	0.15	99.4 (0.10)*	*E. globulus*	0.15	52.8 (6.97)**
B. americana	0.25	95.5 (0.61)*	*L. guatemalensis*	0.20	99.5 (0.08)*	*P. guajava*	0.15	70.6 (7.1)***
A. graveolens	0.40	96.3 (0.92)*	*C. lusitanica*	0.25	99.1 (0.03)*	*P. oocarpa*	0.15	75.6 (1.74)*
R. chalepensis	0.50	96.1 (0.25)***	*A. millefolium*	0.25	99.4 (0.10)*	*R. officinalis*	0.20	96.1 (0.61)*
T. filifolia	0.70	60.6 (5.75)*	*O. basilicum*	0.35	98.7 (0.09)*	*B. orellana*	0.25	96.2 (0.45)*
						P. auritum	0.40	96.7 (0.39)*

µl/ml; * p ≤ 0.001; ** p ≤ 0.004; *** p ≤ 0.026.

the tongue and skin cell lines with TIs of 1.25 and 1.11, respectively. This result is significant because only two low IC_{50} values among the 22 species tested occurred against skin cancer cells (Table 2).

Conclusion

This study provided an increased understanding about the activity of essential oils against cancer cell lines and cytotoxicity from medicinal plants commonly used in Guatemala. IC_{50} values indicated that essential oils can be highly effective against one or more cancer cell lines with oils from *C. aurantiifolia*, *L. graveolens*, *O. vulgare*, *R. chalepensis*, and *T. ambrosioides* showing potential for future development.

Results from therapeutic indices and cell proliferation assay consistently indicate that essential oils from *C. limetta, C. aurantium*, *L. graveolens, O. vulgare, E. globulus, R. officinalis,* and *R. chalepensis* were more toxic to cancerous cells than cells from the established cell line which shows broad as well as cell line specific activity. Future research should include identification of active compounds, determining mechanisms of action of these compounds, possible synergistic interactions, and animal and clinical studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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