Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
21(4): 587-593, Jul./Aug. 2011

Chemical composition of essential oils of *Piper jacquemontianum* and *Piper variabile* from Guatemala and bioactivity of the dichloromethane and methanol extracts

Sully M. Cruz,[1] Armando Cáceres,[*,2] Luis Álvarez,[3] Julio Morales,[3] Miriam A. Apel,[4] Amelia T. Henriques,[4] Efraín Salamanca,[5] Alberto Giménez,[5] Yelkaira Vásquez,[6] Mahabir P. Gupta[6]

[1]Escuela de Química Farmacéutica, Facultad de CCQQ y Farmacia, Universidad de San Carlos, Guatemala,
[2]Escuela de Química Biológica, Facultad de CCQQ y Farmacia, Guatemala,
[3]Herbario USCG, Centro de Estudios Conservacionistas, Universidad de San Carlos, Guatemala,
[4]Faculdade de Farmácia, Universidad Federal Rio Grande do Sul, Brasil,
[5]Instituto de Investigaciones Fármaco Bioquímicas, Universidad Mayor de San Andrés, Bolivia,
[6]Centro de Estudios Farmacognósticos de la Flora Panameña, Universidad de Panamá, Panamá.

Abstract: The essential oils from two native species from Guatemala were studied for their chemical composition and the dichloromethane and methanol extracts for their biological activity. A GC-MS analysis of the essential oil from *Piper jacquemontianum* Kunth, Piperaceae, showed 34 constituents, consisting mainly of linalool (69.4%), while *Piper variabile* C. DC. essential oil had 36 constituents, camphor (28.4%), camphene (16.6%) and limonene (13.9%) being the major components. Dichloromethane extracts of both species were cytotoxic against MCF-7, H-460 and SF-268 cell lines (<7 µg/mL). Dichloromethane extract of *P. jacquemontianum* was slightly active against bacteria (0.5 mg/mL), was active against promastigotes of *Leishmania* (20.4-61.0 µg/mL), and epimastigotes of *Trypanosoma cruzi* (51.9 µg/mL). The methanol extract of *P. variabile* showed antimalarial activity against *Plasmodium falciparum* F32 (4.5 µg/mL), and the dichloromethane extract against *Leishmania* (55.8-76.3 µg/mL) and *T. cruzi* (45.8 µg/mL). None of the extracts from the two species was active against *Aedes aegypti* larvae and *Artemia salina* nauplii.

Article

Received 5 Nov 2010
Accepted 21 Jan 2011
Available online 17 Jun 2011

Keywords:
antimicrobial activity
cytotoxic activity
essential oil
extracts
Guatemala
Piper

ISSN 0102-695X
doi: 10.1590/S0102-695X2011005000110

Introduction

Within the context of a multinational Organization of American States sponsored project aimed at studying the potential of Central American biodiversity, species of *Piper* native to Guatemala were studied for the chemical composition of the essential oil, and the biological activity of dichloromethane and methanol extracts.

The genus *Piper* has a large number of species, and has been of worldwide interest due to their wide utilization as aromatic species and their use in the traditional medicine. This genus is of high botanical, chemical and pharmacological complexity (Dyer & Palmer, 2004). The analysis of volatile chemical components of species of *Piper* has shown the presence of monoterpenes, sesquiterpenes and arylpropanoids with interesting insecticidal, antimicrobial, and antioxidant activities (Parmar et al., 1997; Martins et al., 1998; Moreira et al., 1998). The two species collected from Guatemala, locally known as "cordoncillo" (Standley & Steyermark, 1952), were chosen for their ethnomedical use for treatment of infection, anaemia and body aches (Cleaves, 2001; Michel et al., 2007). The study of these species is relevant, as they have not been studied before, have important medicinal properties, and are a potential source of aroma.

Material and Methods

Plant material

Leaves of *Piper jacquemontianum* Kunth and *P. variabile* C. DC., Piperaceae, were collected in Lachuá, Alta Verapaz, botanically identified by one of the authors (JM), and voucher specimens (21304 and 21310, respectively) deposited at the USCG Herbarium at CECON. Plant material (500-900 g) was shade-dried at 35 °C for 5-7 days and ground.

Extraction and analysis of essential oils

Essential oils were obtained by hydrodistillation for 3 h in a modified Clevenger-type apparatus. The yield of the oil was calculated on the basis of dry weight of the plant material. Oil samples were analysed on a Shimadzu Gas Chromatographer (GC-17A) with injector, quantification was achieved by electronic integration by normalization technique. For separation of constituents an apolar Durabond-DB5 column (30 m long and 0.25 mm of I.D.) with polydimethylphenyl siloxane containing 5% of phenyl groups in a 0.25 µm thick film (John Wiley & Sons Scientific, USA); a polar column (LM-120; by L&M, San Carlos, SP, Brazil), filled with propylene glycol; and a flame ionization detector (FID) were used. Conditions of the equipment included: temperature program 60-300 °C, 3 °C/min; analysis time 60 min; injector temperature 220 °C; FID/EM interface: 250 °C; amount of sample injected: 1 µL diluted in diethyl ether. Qualitative analysis was done with the same equipment fitted to a Shimadzu mass spectrophotometer (GC/MS-QP5000), connected with cylindric quadruple and operated by ionization energy of 70 eV, obtained by electronic impact technique. Retention indices were calculated with an aliphatic hydrocarbon series (C8-C22). The chiral isomeric ratios of the main monoterpene components of the essential oil were studied using a Cyclosil B column; temperatures program 60-300 °C, 3 °C/min.

Sequential extraction

Two extracts were obtained by sequential percolation in a stainless steel percolator, first with dichloromethane twice, and then with methanol for three days. The extracts were concentrated under reduced pressure at a temperature <40 °C in a rotary evaporator, and kept in a vacuum dryer.

Antimicrobial screening

Activity against bacteria and yeast was determined by an agar plate dilution method according to Mitscher et al. (1972), microbial strains used were *Bacillus subtilis* ATCC 6051, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Mycobacterium smegmatis* ATCC 607, *Candida albicans* ATCC 10231 and *Cryptococcus neoformans* C13. Minimum inhibitory concentration (MIC) was determined by the same procedure in quadruplicate containing agar-extract dilutions.

Artemia salina *cytotoxicity and larvicidal activity*

Artemia assay was done according to Solis et al. (1993) using extract dilutions of 1,000, 500 and 250 µg/mL; LC50 was calculated by non parametric regression analysis using a Finney program for Basic. One, two and three instars larvae (*Aedes aegypti* and *Anopheles albimanus*) were used against the same dilutions, after 24 h, death larvae were evaluated visually (Araujo et al., 2003).

Antiprotozoal activity

Activity was measured on *in vitro* cultures of *Leishmania* parasite in promastigote forms of complex *L. amazonensis* (strand PH8), complex *L. braziliensis* (strand M2903) and complex *L. donovani* (strand PP75), cultivated at 26 °C in Schneider medium supplemented with 5% of inactivated (56 °C x 30 min) calf bovine serum. Parasites in logarithmic phase of growth, at a concentration of 1x106 parasites/mL, were distributed on 96 micro well plates with different concentration of the substances (100, 50 and 25 µg/mL), and incubated for 72 h. Activity was determined by optical counting in an inverted microscope. Pentamidine (100 µg/mL) was used as reference drug during the evaluations that were done by triplicate. Under the same conditions, epimastigote forms (1x104 parasites/mL) of *Trypanosoma cruzi* (strand Tulahuen), were cultivated in LIT medium supplemented with 5% calf bovine serum. Amphotericin B (10 µg/mL) was used as reference drug (Fornet et al., 1994). The IC50 of the active extract was determined by lineal interpolation of a typical curve obtained by plotting the logarithm of the concentration vs. the % of inhibition, using programs for logit, probit or polynomial regression analysis. *Plasmodium falciparum* F32 (Indochine, clone W2, chloroquine sensitive strain) was cultivated in glucose-enriched RPMI 1640 medium, supplemented with 10% human serum. Viability was measured by fluorochrome (PicoGreen®) intercalation and read in a micrometric fluorometer according to Corbett et al. (2004).

Neoplastic cell lines cytotoxicity

Human cancer cell lines from breast (MCF-7),

lung (H-460) and central nervous system (SF-268) were kindly donated by National Cancer Institute, USA, cultivated *in vitro* and the bioassay performed according to the standard procedure described by Monks et al. (1991; 1997), using adriamycin as reference drug.

Results

The yields of the essential oils obtained from leaves of *P. jacquemontianum* and *P. variabile* by hydrodistillation were 0.8 and 0.7% respectively. The GC/MS analysis of *P. jacquemontianum* showed at least 34 constituents, linalool (69.4%), (*E*)-nerolidol (8.0%), and α-pinene (3.2%) being the major constituents while *P. variabile* showed at least 36 constituents, mainly camphor (28.4%), camphene (16.6%), limonene (13.9%), and *o*-cymene (6.3%) (Table 1). In the chiral characterization, linalool, camphene and limonene had strong chiral isomeric preference, with more than 84% for (+)-linalool and 94% of (+)-limonene form, while

Table 1. Volatile components identified in essential oils of *Piper jacquemontianum* and *P. variabile*, Piperaceae.

Components	RT-MS	*KI-DB5	*Piper jacquemontianum* %	*Piper variabile* %
Tricyclene	6.655	908	-	0.2
α-Pinene	7.037	918	3.2	2.9
Camphene	7.583	933	0.1	16.6
β-Pinene	8.515	958	2.4	0.7
Myrcene	9.048	973	0.4	0.3
α-Phellandrene	9.569	987	-	0.2
o-Cymene	10.426	1008	0.1	6.3
1,8-Cineole	10.623	1013	1.4	-
Limonene	10.659	1011-1014	0.7	13.9
(*Z*)-β-Ocimene	10.937	1020	0.2	-
(*E*)-β-Ocimene	11.388	1030	2.5	-
(*Z*)-Linalool oxide	12.444	1054	0.3	-
Terpinolene	13.131	1070	0.2	-
Linalool	14.000	1089	69.4	-
Camphor	15.785	1128	-	28.4
(*Z*)-β-Terpineol	15.905	1130	-	1.4
Isoborneol	16.675	1147	-	0.3
Non identified	16.861	1150	-	0.4
Terpinen-4-ol	17.829	1162	0.1	-
α-Terpinol	17.829	1176	1.1	-
(*E*)-β-Geraniol	20.843	1237	0.2	-
Bornyl acetate	22.139	1267	0.3	0.5
(*E*)-Linalool oxide acetate	22.225	1269	0.4	-
(*E*)-Sabinyl acetate	22.778	1281	0.1	-
p-Cymen-7-ol	22.683	1279	-	1.5
Thymol	22.801	1282	-	0.3
3'-Methoxy-acetophenone	23.105	1289	-	2.2
Non identified	24.269	1315	-	0.3
Geranyl acetate	26.540	1364	1.6	-
β-Caryophyllene	28.100	1398	0.4	2.3
α-Humulene	29.523	1432	0.2	-
Seychellene	29.863	1440	-	2.7
γ-Muurolene	30.513	1456	0.1	-
Germacrene D	30.694	1460	1.0	1.0
β-Selinene	31.312	1475	0.1	0.3
Viridiflorene	31.278	1474	0.3	-
α-Muurolene	31.498	1479	0.2	-

δ-Cadinene	32.431	1502	0.3	-
Elemicin	33.793	1538	-	0.4
(*E*)-Nerolidol	34.120	1546	8.0	1.8
Spathulenol	34.676	1561	-	1.4
Caryophyllene oxide	34.844	1566	-	2.2
Guaiol	35.552	1584	-	6.3
Humulene oxide I	35.665	1587	-	0.7
Non identified	36.656	1614	-	0.3
γ-Eudesmol	36.749	1616	0.3	-
τ-Cadinol	37.092	1626	0.1	-
α-Muurolol	37.154	1627	-	0.2
Cubenol	37.325	1632	0.2	0.7
β-Eudesmol	37.458	1636	-	0.3
Non identified	37.571	1639	-	1.8
α-Eudesmol	37.606	1640	1.0	-
Non identified	38.283	1658	0.4	-
Bulnesol	38.108	1653	-	0.2
Non identified	38.822	1673	-	0.3
Non identified	39.033	1678	-	0.4
(*E*)-Nerolidol acetate	40.161	1709	2.2	-
Nonadecanal	53.269	2100	-	0.7
Identified constituents (%)			99.5	96.5

*Kovats index on DB-5 column.

camphene showed the preference for the (-)-camphene form. For camphor, only a racemic mixture could be observed.

Cytotoxic activity against MCF-7, H-460 and SF-268 cell lines was very good in both of the dichloromethane extracts (<7 µg/mL). The methanol extract of *P. jacquemontianum* showed activity against promastigotes of *L. amazonensis* (61 µg/mL), *L. braziliensis* (20.4 µg/mL) and *L. donovani* (20.8 µg/mL), and epimastigotes of *T. cruzi* (51.9 µg/mL), while the dichloromethane extract showed activity against *L. amazonensis* and *L. braziliensis* at a higher concentration (102.8 µg/mL). The methanol extract of *P. variabile* showed good activity against *P. falciparum* F32 (4.5 µg/mL), and the dichloromethane extract was active against *L. donovani* (55.8 µg/mL), *L. braziliensis* (66.3 µg/mL), *L. amazonensis* (76.3 µg/mL) and *T. cruzi* (45.8 µg/mL).

The dichloromethane extract showed little activity against *B. subtilis* and *M. smegmatis*; no activity was shown against *A. aegypti, A. albimanus* larvae and against *A. salina* nauplii (Table 2).

Discussion

The literature on the genus *Piper* shows a great variability in chemical composition among different species, for Latin-American species the main constituents reported are monoterpenes and sesquiterpenes, while African and Asian species are rich in phenylpropanoids (Dyer & Parmar, 2004). The main constituents described in several organs from *Piper* species are: camphor, apiole, myristicin, safrole, sarisan, dilapiole, linalool, α-pinene, α-humulene, β-caryophyllene, as well as alkaloids, lignans, terpenes, steroids, propenyphenols, kavapyones, chalcones, flavonoids and piperolides (Von Poser et al., 1994; Martins et al., 1998; Parmar et al., 1997; Moreira et al., 1998).

The linalool content in *P. jacquemontianum* (69.4%) is the highest reported for any *Piper* species and could be a valuable resource for perfumery industry. Mundina et al. (1997) showed the presence of linalool in three species from Panama (*Piper fimbriulatum* C. DC., *Piper arboreum* Aublet and *Piper obliquum* Ruiz & Pavón), and Guerrini et al. (2009) from *Piper aduncum* L. from Ecuador, although their contents were low. All four species from Saint Tomé and Príncipe had low amount, particularly *Piper capense* L. and *Piper guineense* Schumach. & Thonn. (Martins et al., 1998). From three species from Cameroon, *Piper umbellatum* L. contained linalool (14.4%) as major constituent (Françoise et al. 2009). In Brazil, from seven species, only *Piper goesii* Yunck showed traces of linalool

Table 2. Bioactivity of extracts from *P. jacquemontianum* and *P. variabile*, Piperaceae (µg/mL).

Bacteria	*P. jacquemontianum*		*P. variabile*	
	CH_2Cl_2	MeOH	CH_2Cl_2	MeOH
Bacillus subtilis ATCC 6051	500	>1000	>1000	1000
Escherichia coli ATCC 25922	>1000	>1000	>1000	>1000
Mycobacterium smegmatis ATCC 607	500	>1000	>1000	1000
Pseudomonas aeruginosa ATCC 27853	>1000	>1000	>1000	>1000
Salmonella typhi ATCC 14028	>1000	>1000	>1000	>1000
Staphylococcus aureus ATCC 25923	>1000	>1000	>1000	>1000
Yeast				
Candida albicans ATCC 10231	>1000	>1000	>1000	>1000
Cryptococcus neoformans FCQ C-13	>1000	>1000	>1000	>1000
Protozoa				
Leishmania amazonensis PH 8 (promastigotes)	>1000	61	76.3	>1000
L. braziliensis M 2903 (promastigotes)	102.8	20.4	66.3	>1000
L. donovani PP 75 (promastigotes)	102.8	20.8	55.8	>1000
Plasmodium falciparum F32 chloroquine-sensitive	>1000	>1000	>1000	4.5
Trypanosoma cruzi Tulahuen C4 (epimastigotes)	>1000	51.9	45.8	>1000
Larvicidal				
Artemia salina nauplii	>1000	>1000	>1000	>1000
Aedes aegypti larvae	>1000	>1000	>1000	>1000
Anopheles albimanus larvae	>1000	>1000	>1000	>1000
Cytotoxicity to cancer cell lines				
H-460	4.7	>10	7.1	>10
MCF-7	4.6	>10	6.3	>10
SF-268	4.1	>10	4.7	>10

Controls. Amphotericin B IC50: 0.2 µg/mL; Pentamidine IC50: 10 µg/mL; Chloroquine IC: 32 nM, Adryamicin GI50: MCF-7: 6.2x10-7, H-460: 3.6x10-7, SF-268: 5.3x10-7.

(Dias dos Santos et al., 2001); Constantin et al. (2001) showed that myrcene (52.6%) and linalool (15.8%) were the major constituents of the essential oil of leaves of *Piper regnelli* (Miq.) C. DC.; de Almeida et al. (2009) showed that linalool was the major constituent of the essential oil of leaves from *Piper divaricatum* G. Mey. (23.4-29.7%); and, Autran et al. (2009) showed low content in the leaf of *Piper marginatum* Jacq.

The presence of camphor as a major constituent in *P. variabile* (28.4%) is noteworthy, since previous studies showed from four species from Saint Tomé and Príncipe, and Ecuador, the essential oils from *P. guineense* (0.8%) (Martins et al., 1998), *P. obliquum* (0.25%) and *P. aduncum* (0.05%) had very little amount (Guerrini et al., 2009).

The main bioactivity demonstrated was the cytotoxicity against cancer cell lines. In a previous study, Calderon et al. (2006) reported cytotoxicity in the whole ethanol extract of *P. jacquemontianum*, however, the cytotoxicity in dichloromethane fraction of *P. variabile* is shown for the first time.

Cytotoxic activity has been demonstrated in other *Piper* species. Fractions and compounds from *Piper hostmannianum* var. *berbicense* (Miq.) C. DC. showed no cytotoxic activity against MCF-7 cell lines (Portet et al., 2007); ethanol extract of Piper sarmentosum Roxb. inhibited HepG2 cells and non-malignant Chang's liver cell lines (Ariffin et al., 2009); dichloromethane and methanol extracts from fruits of *Piper nigrum* L. and *Piper chaba* Hunter were inactive against C32 and HeLa cell lines (Atjanasuppat et al., 2009); although, *E*-piplartine isolated from roots of *P. chaba* showed cytotoxicity against several cell lines (BC-8, PCC4, P388S1, IMR32), significantly increased by curcumin (Jyothi et al., 2009). Essential oil from leaves of *Piper gaudichaudianum* Kunth was cytotoxic to Chinese hamster lung fibroblast (V79) cells (Péres et al., 2009).

With respect to antiprotozoal activity, in a previous study we demonstrated that the whole ethanol

extract of *P. jacquemontianum* was active against *P. falciparum* (IC50 12 µg/mL), *T. cruzi* (IC50 12 µg/mL), and *L. mexicana* (IC50 22 µg/mL) (Calderon et al., 2010), in this study we were not able to confirm the activity against *P. falciparum*, but the other activities were confirmed in dichloromethane and methanol extracts. In the case of *P. variabile* it is the first time that the activity against *Leishmania* and *T. cruzi* is reported.

The antiprotozoal activity of both species is interesting, although other species have shown similar activity. From twelve extracts from three species of *Piper* from Comores, only the dichloromethane extract of *P. capense* showed activity against P. falciparum W2 (7 µg/mL) (Kaou et al., 2008). By bioassay-guided purification from hexane extract of *P. hostmannianum* var. *berbicense in vitro* activity to *P. falciparum* (IC50 5.64 µM) and *in vivo* activity against *Plasmodium* vinckei petteri was demonstrated by (-)-methyllinderatin (Portet et al., 2007). From 94 species detected by ethnobotany in Peru, eight species of *Piper* were detected, *P. aduncum* showed activity against *P. falciparum* (9.6±1.7 µg/mL), and *Piper dennisii* Trel. against *L. amazonensis* (IC50 10±0.15 µg/mL) (Valadeau et al., 2009).

Activity against bacteria and yeast is considered small, since only activity against *B. subtilis* and *M. smegmatis* were demonstrated at a high MIC. No activity was demonstrated against insect larvae as well as against *A. salina* nauplii. Other species have shown larvicidal activity against *A. aegypti*, such as *P. fimbriulatum* (Solis et al., 2005) and *P. marginatum* (Autran et al., 2009).

It is evident that the two species or *Piper* native to sub-tropical Guatemala are potential resources for development of new crops that might help in the search for alternative products. Further studies are being conducted for bioguided evaluation of these extracts in order to determine the compounds responsible for such activities, as well as agrotechnological development for sustainable utilization of this native resource.

Acknowledgements

The authors thank the financial support of General Directorate of Research (DIGI) at USAC, Guatemala, the Organization of American States (OAS, grant SEDI/AICD/106/01), and the cooperation within RIBIOFAR (Red 0284) Network of the Ibero-American Program of Science and Technology for Development (CYTED). The support of Ricardo Véliz MSc, School of Chemistry, USAC, and the technical assistance of Ana Gómez and Vinicio García are kindly appreciated.

References

Araujo EC, Silveira ER, Lima MA, Neto MA, de Andrade IL, Santiago GM, Mesquita AL 2003. Insecticidal activity and chemical composition of volatile oils from *Hyptis martiusii* Benth. *J Agric Food Chem 51*: 3760-3762.

Ariffin SHZ, Haryani WH, Ariffin ZZ, Safian MF, Senafi S, Wahab RMA 2009. Intrinsic anticarcinogenic effects of *Piper sarmentosum* ethanolic extract on a human hepatoma cell line. *Cancer Cell Int 9*: 6 (doi:10.1186/1475-2867-9-6).

Atjanasuppat K, Wongkham W, Meepowpan P, Kittakoop P, Sobhon P, Bartlett A, Whitfield PJ 2009. In vitro screening for anthelmintic and antitumor activity of ethnomedicinal plants from Thailand. *J Ethnopharmacol 123*: 475-482.

Autran ES, Neves IA, da Silva CSB, Santos GKN, da Câmara CAG, Navarro DMAF 2009. Chemical composition, oviposition deterrent and larvicidal activities against *Aedes aegypti* of essential oils from *Piper marginatum* Jacq. (Piperaceae). *Biores Technol 100*: 2284-2288.

Calderon AI, Vazquez Y, Solis PN, Caballero-George C, Zacchino S, Gimenez A, Pinzon R, Caceres A, Tamayo G, Correa M, Gupta MP 2006. Screening of Latin American plants for cytotoxic activity. *Pharm Biol 44*: 130-140.

Calderon AI, Romero LI, Ortega-Barría E, Solis PN, Zacchino S, Gimenez A, Pinzon R, Caceres A, Tamayo G, Guerra C, Espinosa A, Correa M, Gupta MP 2010. Screening of Latin American plants for antiparasitic activities against malaria, Chagas disease, and leishmaniasis. *Pharm Biol 48*: 545-553.

Cleaves C 2001. *Etnobotánica participativa en siete comunidades de la zona de influencia del Parque Nacional Laguna Lachúa, Cobán, Alta Verapaz*. Guatemala, 282p. Tesis, Escuela de Biología, Universidad de San Carlos.

Constantin MB, Sartorelli P, Limberger R, Henriques AT, Steppe M, Ferreira MJ, Ohara MT, Emerenciano VP, Kato MJ 2001. Essential oil from *Piper cernuum* and *Piper regnellii*: antimicrobial activities and analysis by GC/MS and ^{13}C-NMR. *Planta Med 67*: 771-773.

Corbett Y, Herrera L, González J, Cubilla L, Capson T, Coley P, Kursar T, Romero L, Ortega-Barría E 2004. A novel DNA based microfluorimetric method to evaluate anti-malarial drug activity. *Am J Trop Med Hyg 70*: 119-124.

De Almeida JGL, Silveira ER, Pessoa ODL, Nunes EP 2009. Essential oil composition from leaves and fruits of *Piper divaricatum* G. Mey. *J Essent Oil Res 21*: 228-230.

Dias dos Santos PR, de Lima Moreira D, Guimarães EF, Coelho Kaplan MA 2001. Essential oil analysis of 10 Piperaceae species from the Brazilian Atlantic coast. *Phytochemistry 58*: 547-551.

Dyer LA, Palmer APDN 2004. *Piper: A Model Genus for Studies of Phytochemistry, Ecology, and Evolution*. New York: Kluwer Academic/Plenum Publishers, 214p.

Fournet A, Angelo A, Muñoz V 1994. Leishmanicidal and trypanocidal activities of Bolivian medicinal plants.

J Ethnopharmacol 41: 19-37.

Françoise T, Michel JDP, Lambert SM, Mdifor F, Vyrym WNA, Henri AZP, Chantal M 2008. Comparative essential oil composition and insecticidal effect of different tissues of *Piper capense* L., *Piper guineense* Schum. et Thonn., *Piper nigrum* L. and *Piper umbellatum* L. grown in Cameroon. *Afr J Biotech 8*: 424-431.

Guerrini A, Saccheti G, Rossi D, Paganetto G, Muzzoli M, Andreotti E, Tognolini M, Maldonado ME, Bruni R 2009. Bioactivities of *Piper aduncu*m L.and *Piper obliquum* Ruiz & Pavon (Piperaceae) essential oil from Easter Ecuador. *Environ Toxicol Pharmacol 27*: 39-48.

Jyothi D, Vanathi P, Mangalo Gowri P, Rao VRS, Rao JM, Sreedhar AS 2009. Diferuloylmethane augments the cytotoxic effect of piplartine isolated from *Piper chaba. Toxicol In Vitro 23*: 1085-1091.

Kaou AM, Mahiou-Leddet V, Hutter S, Aïnouddine S, Hassani S, Yahaya I, Azas N, Ollivier E 2008. Antimalarial activity of crude extracts from nine African medicinal plants. *J Ethnopharmacol 116*: 74-83.

Martins AP, Salguero L, Vila R, Tomi F, Cañigueral S, Casanova J, Proença da Cunha A, Adzet T 1998. Essential oils from four *Piper* species. *Phytochemistry 49*: 2019-2023.

Michel JL, Duarte RE, Bolton JL, Huang Y, Cáceres A, Veliz M, Soejarto DD, Mahady GB 2007. Medical potential of plants used by the q'eqchi maya of Livingston, Guatemala for the treatment of women's health complaints. *J Ethnopharmacol 114*: 92-101.

Mitscher LA, Leu RP, Bathala MS, Wu WN, Beal JL, White R 1972. Antimicrobial agents from higher plants. I. Introduction, rationale and methodology. *Lloydia 35*: 157-166.

Monks A, Scudeiro DA, Skehan P, Shoemaker R, Pauli K, Vistica D, Hosse C, Langley J, Cronise P, Vaifgro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J, Boyd M 1991. Feasibility of high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Nat Cancer Inst 83*: 757-766.

Monks A, Scudeiro DA, Jonson GS, Pauli KD, Sausville EA 1997. The NCI-anticancer drug screen: A smart screen to identify effectors of novel targets. *Anti-cancer Drug Des 12*: 533-541.

Moreira DL, Guimarães EF, Kaplan MAC 1998. Essential oil analysis of two *Piper* species (Piperaceae). *An Acad Bras Cien 70*: 751-754.

Mundina M, Vila R, Tomi F, Gupta MP, Adzet T, Casanova J, Cañigueral S 1997. Leaf essential oils from three Panamenian *Piper* species. *Phytochemistry 47*: 1277-1282.

Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen CE, Boll PM 1997. Phytochemistry of the genus *Piper. Phytochemistry 46*: 597-673.

Péres VF, Moura DJ, Sperotto ARM, Damasceno FC, Caramão EB, Zini CA, Saffi J 2009. Chemical composition and cytotoxic, mutagenic and genotoxic activities of the essential oil from *Piper gaudichaudianum* Kunth leaves. *Food Chem Toxicol 47*: 2389-2395.

Portet B, Fabre N, Roumy V, Gornitzka H, Bourdy G, Chevalley S, Sauvin M, Valentin A, Moulis C 2007. Activity-guided isolation of antiplasmodial dihydrochalcones and flavanones from *Piper hostmannianum* var. *berbicense. Phytochemistry 68*: 1312-1320.

Solis PN, Olmedo D, Nakamura N, Calderon AI, Hattori M, Gupta MP 2005. A new larvicidal lignan from *Piper fimbriulatum. Pharm Biol 43*: 378-281.

Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson D 1993. A microwell cytotoxicity assay using *Artemia salina* (Brine shrimp). *Planta Med. 59*: 250-252.

Standley PC, Steyermark JA 1952. Flora of Guatemala. Fieldiana: *Botany 24*: 284-285, 333-334.

Valadeau C, Pabon A, Deharo E, Albán-Castillo J, Estevez Y, Lores FA, Rojas R, Gamboa D, Sauvin M, Castillo D, Bourdy G 2009. Medicinal plants from the Yanesha (Peru): Evaluation of the leishmanicidal and antimalarial activity of selected extracts. *J Ethnopharmacol 123*: 413-422.

Von Poser GL, Rórig LR, Henriques AT, Limaty G, Menut C, Bessiere JM 1994. Aromatic plants from Brazil. III. The chemical composition of *Piper gaudichaudianum* Kunth. and *Piper mikanianum* (Kunth.) Stendel essential oils. *J Essent Oil Res 6*: 337-340.

***Correspondence**

Armando Cáceres

Escuela de Química Biológica, Facultad de CCQQ y Farmacia,
Universidad de San Carlos, Edificio T-11
Ciudad Universitaria zona 12, Guatemala
caceres-armando@usac.edu.gt
Tel.: 502 2418 9410
Fax: 502 2418 9414