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Chemical Composition and Antibacterial activity of Essential Oils from Fruits of *Vismia baccifera* and *Vismia macro*phylla Collected at different Locations in Venezuelan Andes

Alexis Buitrago [a,b], Janne Rojas [b*], Judith Velasco [c], Marielba Morillo [d], Nicolas Joly [e], Luis Rojas [a] and Patrick Martin [e*]

[a] Department of Analysis and Control, Faculty of Pharmacy and Bioanalysis, University of Los Andes, 5101, Mérida, Venezuela.
[b] Organic Biomolecular Research Group, Research Institute, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida 5101, Venezuela.
[c] Department of Microbiology and Parasitology, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida 5101, Venezuela.
[d] Ecology and Nutrition Research Group, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida 5101, Venezuela.
[e] Unité Transformations and Agroressources, ULR7519, Université d'Artois - UniLaSalle, F-62408 Bethune, France.

Authors' contributions

This work was carried out in collaboration among all authors. Author JR designed the research study. Authors AB, LR and NJ performed the research. Author JR, AB and MM wrote the manuscript. Authors LR, JV and PM performed formal analysis research. Author AB performed the statistical analysis. Author NJ and PM provided writing-revision. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: janne.rojas24@gmail.com; patrick.martin@univ-artois.fr;*

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ABSTRACT

Background: Genus *Vismia* (Hipericaceae/Clusiaceae) is distributed mainly in tropical and subtropical areas of Central America and South America although some species have been reported in Africa as well. Species of this genus have been used in traditional medicine to alleviate different ailments such as skin infections, to treat urinary tract disorders, as antirrheumatic, antipyretic, among others. Previous investigations have revealed that species from *Vismia* genus are composed mainly of terpenes, anthrones, lignans, flavonoids, anthraquinones, steroids and xanthones. The purpose of the investigation was to determine the chemical composition and antimicrobial activity of essential oils from the fruits of *V. baccifera* and *V. macrophylla* collected in Venezuelan Andes.
Methods: *Vimia* species were collected at different locations in Mérida and Táchira state, Venezuela. Essential oils were obtained through Hidrodistilation and chemical compositon was performed by GC and GC/MS techniques. Antimicrobial analysis was carried out by disc diffusion assays.
Results: GC and GC/MS analysis showed that these species are mainly composed of sesquiterpenes being *α*-curcumene, *β*-curcumene, germacrene D, *γ*-bisabolene and *β*-caryophyllene among the major components. Antimicrobial activity was also performed with species under investigation. Results showed a broad spectrum of activity since both species were able to inhibit not only Gram- positive (*Stpahylococcus aureus, Enterococcus faecalis*) and Gram-negative bacterial strains (*Escherichia coli, Pseudomonas aeruginosa*) but yeast (*Candida albicans and Candida krusei*) as well.
Conclusions: According to the results observed in the investigation, *Vismia* species might be considered as an alternative to aid infectious diseases.

Keywords: Vismia macrophylla; Vismia baccifera; essential oil; β-curcumene; germacrene-D; β-caryophyllene; antimicrobial activity.

1. INTRODUCTION

The Vismieae tribe belongs to the Hypericaceae or Clusiaceae family and is represented by three genera: *Vismia*, *Harungana* and *Psorospermum*; they occur as shrubs or trees, some reaching a height of 25 m, and grow in tropical and subtropical regions [1,2].

"The *Vismia* Vand. genus is presented as small trees and shrubs found in tropical and subtropical areas of Central America and South America including the countries of Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panamá, Peru, Suriname, Trinidad-Tobago and Venezuela, although some species of this genus have been reported in some areas of Africa as well" (Fig. 1) [2-4].

"In this regard, *Vismia* species have been accommodated in two subgenera: *Vismia* subg. *Vismia*, comprising the American species, and *Vismia* subg. *Afrovismia*, which includes the African species" [5]. "In Venezuela 17 species of *Vismia* have been located being *Vismia baccifera* Triana & Planch and *Vismia macrophylla* Kunth the most common and well-distributed around the country" [6-9].

"Previous investigations have reported 161 compounds isolated from different *Vismia* species, being monoterpenes, sesquiterpenes, triterpenoids, prenylated anthrones, lignans, sterols, flavonoids, flavonols, anthraones, anthraquinones, bianthraquinones, benzophenones, steroids and xanthones, among the most common isolated and characterized components" (Fig. 2) [4,8,11].

Regarding the pharmacological activities, several investigations carried out with different *Vismia* species have revealed antibacterial, antifungal, antiparasitic, insecticidal, antiviral, and anticancer activity. These findings support the traditional medicine where *Vismia* species has

been used to treat skin diseases, such as herpes, dermatitis, leprosy, syphilis, scabies and eczema, among others [4,9-12].

Vismia macrophylla Kunth (Gutifferae/ Clusiaceae) is an Amazonian tree that grows mainly in Central and South America [2,13]. This species has been used in traditional medicine for the treatment of fungal and skin infections, dermatosis, to heal a condition known as “carate” and problems related to vision [14-16].

On the other hand, *Vismia baccifera* (L.) Planch. & Triana, a species typically found in the Amazonian rainforest [17], is commonly used by indigenous populations as purgative, to treat urinary tract disorders, to protect against snake bites, skin diseases, as antirrheumatic, antipyretic, for the treatment of infected wounds and also as a mouth-wash and for women’s douche [18-20].

“Essential oils have also been studied on *Vismia* species; from these, several monoterpenes and sesquiterpenes have been reported, being germacrene-*D*, *α/β*-selinene, *α*-cadinol, *δ*-cadinene, valencene, *β*-elemene, *α*-humulene and *β*-caryophyllene the most commonly detected” [21-25].

A more recent study carried out with the resin of *V. macrophylla* collected from the Peruvian Amazon revealed that the oil is composed mainly of terpen-4-ol (36.08%), *α*-terpineol (21.46%), *α*-eudesmol (16.89%), *α*-copaene (8.89%) and camphor (3.99%) [13].

Another study carried out with the essential oil from leaves and fruits of *Vismia* macrophylla showed the antibacterial activity of these oils against *Staphylococcus aureus* [21], which might be related to their popular use of this species for the treatment of skin infections [4,15]. In addition, essential oil from fruits of *V. baccifera* proved to be effective against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [23].

The present investigation aims to determine the chemical composition and antibacterial activity of essential oils from the fruits of *V. baccifera* and *V. macrophylla* collected at different locations in the Venezuelan Andes.

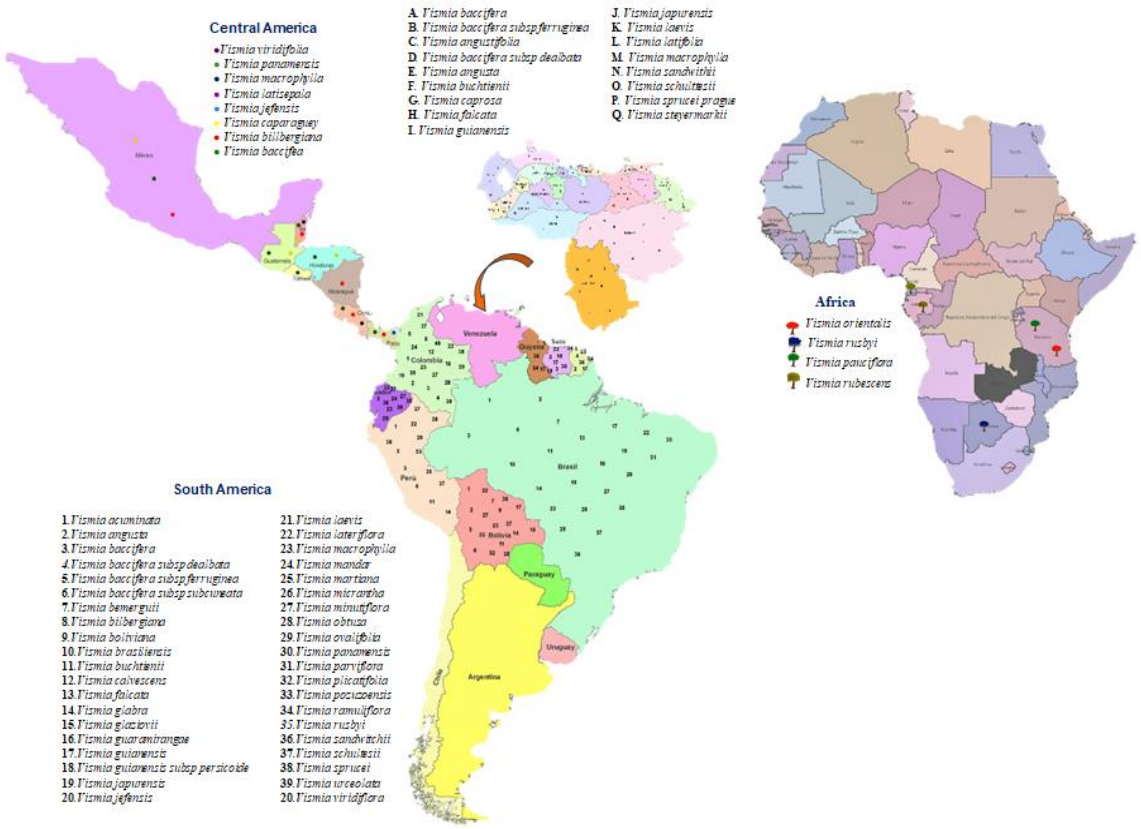


Fig. 1. Distribution of *Vismia* genus in Central America, South America and Africa

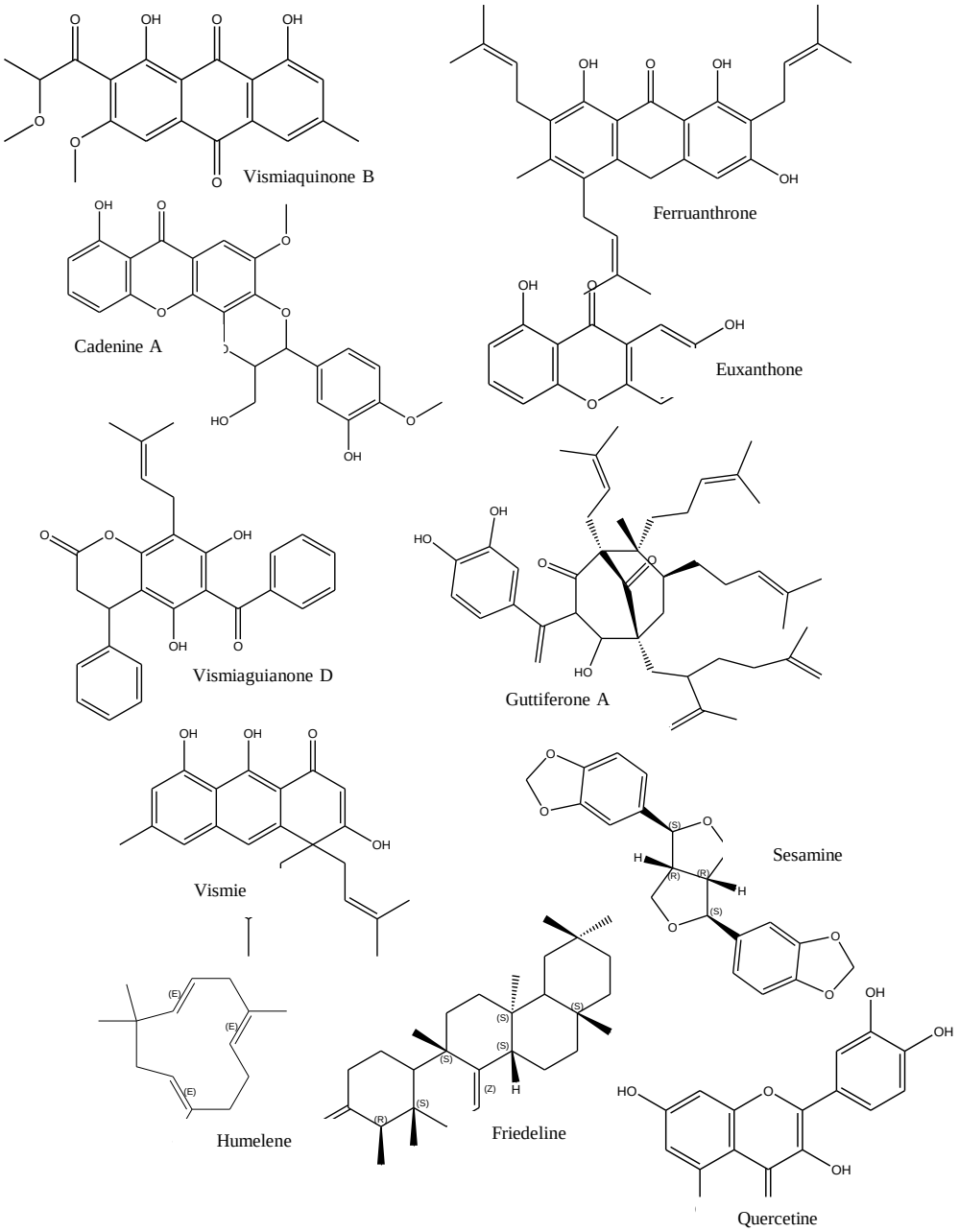
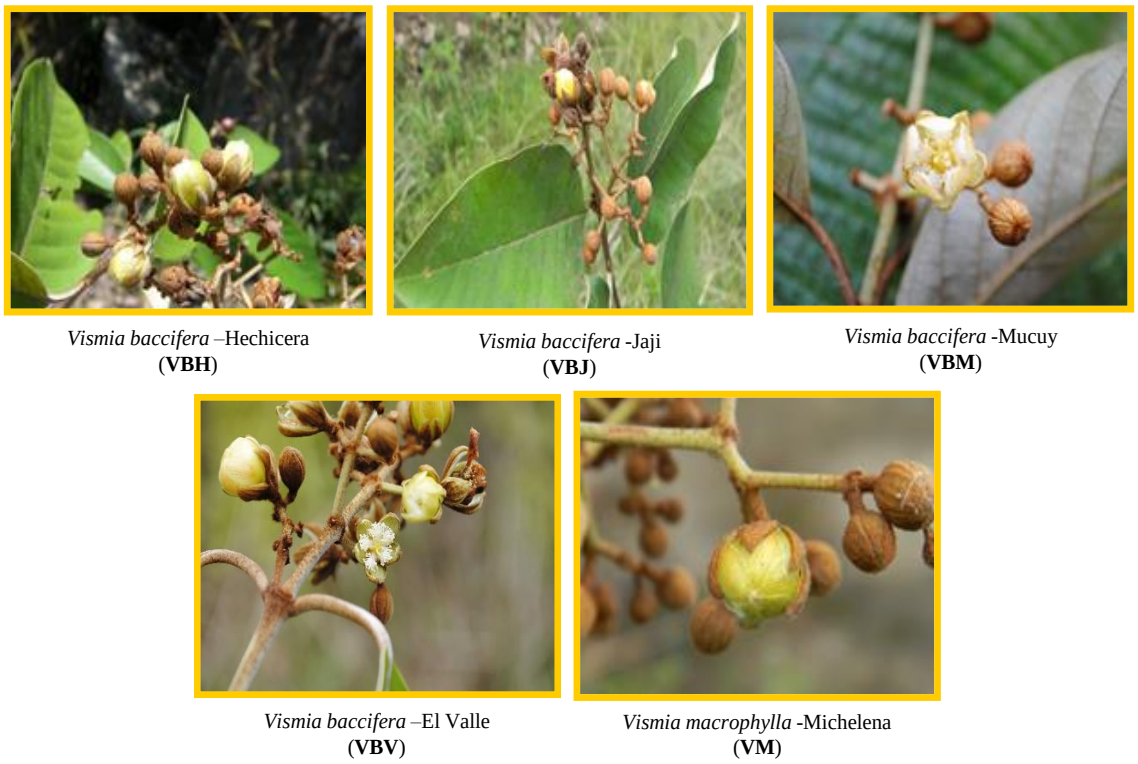


Fig. 2. Secondary metabolites most commonly isolated from different species of *Vismia* genus

2. MATERIALS AND METHODS

2.1 Plant Material

V. macrophylla (VM) was collected from Michelena, Táchira state, at 200 m.a.s.l. (7°56'30" N & 72°14'33" W), whereas *V. baccifera* (VB) was harvested from different locations in Mérida state (Fig. 3): La Hechicera (VBH) at 1989 m.a.s.l. (8º37'37" N-71º09'40" W), Jaji (VBJ) at 1709 m.a.s.l. (8°34'54" N & 71°18'07" W), Mucuy (VBM) at 2095 m.a.sl. (8º37'38" N-71º02'45" W) and El Valle (VBV) at 1835 m.a.s.l. (8°38'13" N & 71°07'37" W) . "These species were collected in February 2017, during the rainy season and flowering stage. Botanical identification was carried out by Dr. Pablo Meléndez, MERF Herbarium, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela. Voucher specimens were deposited under the following codes: VM-JR39, VBH-JR25, VBJ-JR47, VBM-JR54 and VBV-JR51" [26].



Vismia baccifera –Hechicera (**VBH**)

Vismia baccifera -Jaji (**VBJ**)

Vismia baccifera -Mucuy (**VBM**)

Vismia baccifera –El Valle (**VBV**)

Vismia macrophylla -Michelena (**VM**)

Fig. 3. *Vismia bacciera* and *Vismia macrophylla* species collected at different locations in Venezuela Andean

2.2 Isolation of Essential Oils

fresh fruits (F) of VBH (1926 g), VBJ (960 g), VBV (460 g), VBM (390 g) and VM (350 g) were cut into small pieces and subjected, separately, to hydrodistillation for 4h, using a Clevenger-type apparatus. The oils, VBH: 5.6 mL (0.81% w/v); VBJ: 8.5 mL (0.90% w/v); VBV: 4.0 mL (0.87% w/v); VBM: 0.3 mL (1.36% w/v) and VM: 2.0 mL (0.57% w/v) were dried over anhydrous sodium sulfate and stored at 4°C until the analyses were performed.

2.3 Gas Chromatography (GC)

"GC analyses were performed on a Perkin-Elmer AutoSystem gas chromatograph equipped with flame ionization detectors. Two capillary columns of different polarities were used: a 5% phenylmethyl polysiloxane fused-silica column (AT-5, Alltech Associates Inc., Deerfield, IL) (60 m × 0.25 mm, film thickness 0.25 μm) and a polyethylene glycol fused-silica column (AT-WAX, Alltech Associates Inc., Deerfield, IL) of the same dimensions. The initial oven temperature was 60°C; it was then heated to 260°C at 4°C/min and the final temperature was maintained for 20 min. The injector and detector temperatures were 200°C and 250°C, respectively. The carrier gas was helium at 1.0 mL/min and the sample was injected using a split ratio of 1:100. Retention indices were calculated relative to C_8-C_{24} n-alkanes, using only the AT-5 capillary column and comparing values reported in the literature" [27,28].

2.4 Gas Chromatography-Mass Spectrometry (GC-MS)

"GC-MS analyses were carried out on a Hewlett Packard GC-MS system, Model 5973, fitted with a 30 m long, crosslinked 5% phenylmethyl siloxane (HP-5MS, Hewlett Packard, USA) fused-silica column (0.25 mm, film thickness 0.25 μm). The following conditions were applied: source temperature 230°C; quadrupole temperature 150°C; carrier gas helium, adjusted to a linear velocity of 34 m/s; ionization energy, 70 eV; scan range 40-500 amu; 3.9 scans/s. The injected volume was 1.0 μl of a 2% dilution of oil in n-heptane. A Hewlett-Packard ALS injector was used with a split ratio of 1:100. The identification of the oil components was based on the Wiley Registry of Mass Spectral Data (6th Ed.) and NIST 05 data base library, followed by comparisons of mass spectral (MS) data with

published literature and the retention index calculation" [27].

2.5 Bacterial Strains

The microorganisms used for the antimicrobial method were *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25992), *Klebsiella pneumoniae* (ATCC 23357), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (CDCB 385) and *Candida krusei* (ATCC 6258).

2.6 Antimicrobial Method

The antimicrobial activity was carried out according to the disc diffusion assay described by Velasco et al. [29]. "The strains were maintained in agar conservation at room temperature. Each bacterial inoculum was incubated in 2.5 mL Müeller-Hinton broth (BBLTM®) at 37ºC for 18h. The bacterial inoculum was diluted in sterile 0.85% saline to obtain turbidity visually comparable to McFarland Nº 0.5 standard ($1.5x10^{6-8}$ CFU/mL). Every inoculum was spread over plates containing Mueller-Hinton agar and a paper filter disc (6 mm) saturated with 10 µL of essential oil. The plates were left for 30 min at room temperature and then incubated at 37ºC for 24h" [26].

"Antifungal activity was also evaluated following the disc diffusion methodology described by National Committee for Clinical Laboratory Standards" [30]. "Twenty mL Müeller-Hinton agar (BBLTM®) supplemented with glucose (2%, w/v) and methylene blue (0.5 µg/mL) were mixed with 1 mL of each yeast inoculum and turbidity was adjusted to McFarland Nº 1 ($3x10^{8}$ CFU/mL) standard. The content of Petri dishes was allowed to solidify at room temperature and sterile control was also prepared. The inhibitory zone around the disc was measured and expressed in mm. A positive control was also assayed to check the sensitivity of the tested organisms using the following antibiotics: Linezolid® (10 µg), Vancomycin® (30 µg), Tobramycin® (30 µg), Aztreonam® (10 µg), Cefepime® (75 µg), Ceftazidime® (30 µg), Fluconazole® (100 µg) and Voriconazole® (400 µg/mL)" [26]. A negative control was also included in the test using a filter paper disc saturated with dimethyl sulphoxide (DMSO) to discard any activity of this solvent against the microorganisms assayed. The experiments were repeated twice.

"The minimal inhibitory concentration (MIC) was determined only with microorganisms that displayed inhibitory zones. MIC was determined by dilution of the essential oil in DMSO by pipetting 10 µL of each dilution onto a filter paper disc. Dilutions of the oil within a concentration range of 50-950 µL/mL were also carried out. MIC was defined as the lowest concentration that inhibited the visible bacterial growth" [31]. A negative control was also included in the test using a filter paper disc saturated with DMSO to check possible activity of this solvent against the bacteria assayed. The experiments were repeated twice.

2.7 Statistical Analysis

One-way analysis of variance (ANOVA) was carried out to determine whether there is significant difference for antimicrobial activity either on the oils or within the *Vismia* species under investigation. In event of finding any significant difference, further analysis were performed by using the Duncan´s multiple ranges test. Significance level has been established at $\alpha = 0.10$.

3. RESULTS AND DISCUSSION

Essential oils obtained through hydrodistillation of two *Vismia* species collected from different locations at Venezuela Andes were analyzed by gas chromatography and gas chromatography coupled to mass spectrometry. The chemical profile showed that these oils were composed mainly by sesquiterpenes, where, 64.63% corresponded to cyclic sesquiterpenes; 23.17% oxygenated cyclic sesquiterpenes and a minor occurrence of noncyclic sesquiterpenes, alcohols, aldehydes and esters.

Results also showed (Fig. 4) differences between main components of samples analyzed: VBH was mainly composed by α-curcumene (30.6%) and *β*-curcumene (35.5%); VBJ showed germacrene-*D* (18.0%) and *β*-caryophyllene (45.1%); VBV *β*-curcumene (62.6%); VBM *β*-curcumene (18.3%) and *β*-caryophyllene (18.5%); VM showed *γ*-bisabolene (22.3%) and *α*-selinene (11.6%) as major components.

It is important to mention that *α*-cubebene, *α*-copaene, *α*-humulene and *β*-elemene were present in all oils analyzed, thus, these components could be pointed out as possible chemotaxonomy markers of *Vismia* genus (Table 1).

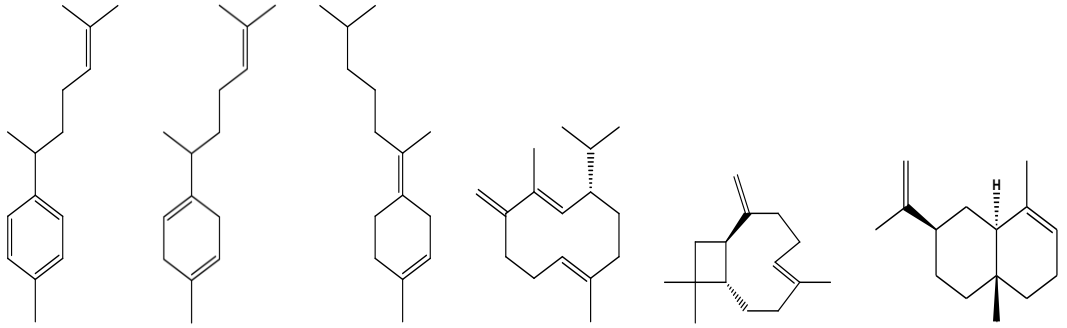


Fig. 4. Major components identified in *V. baccifera* and *V. macrophylla* in present investigation

Table 1. Chemical composition of essential oils of two *Vismia* species collected in different locations in Venezuela Andes

Type sesquiterpene	Compounds	VBH	VBJ	VBV	VBM	VMT	RI*	%PR
Acyclic sesquiterpenes	*β*-farnesene	ND	ND	ND	ND	0.80	1450	VBH, VBJ, VBV y VBM= 0% VMT= (30/ 3; 10%)
	trans-farnesene	ND	ND	ND	ND	1.00	1481	
	(E,E)-*α*-farnesene	ND	ND	ND	ND	2.80	1504	
Monocyclic sesquiterpenes	*δ*-elemene	0.76	ND	ND	ND	ND	1332	VBH= (18/ 7; 38.89%) VBJ= (15/ 5; 33.33%) VBV= (17/ 9; 52.94%) VBM= (16/ 7;43.75%) VMT= (30/ 4;13.33%)
	β-elemene	1.11	1.73	0.61	7.22	0.30	1387	
	α-humulene	0.80	6.69	1.52	3.86	1.00	1451	
	ar-curcumene	30.61	ND	ND	ND	ND	1479	
	germacrene-*D*	ND	18.01	2.48	1.62	12.10	1483	
	α-zingiberene	ND	ND	2.08	ND	ND	1498	
	γ-curcumene	1.55	ND	6.19	1.12	ND	1480	
	germacrene-*A*	ND	2.67	0.80	8.23	ND	1507	
	β-curcumene	35.55	ND	62.58	18.30	ND	1512	
	(Z)-*γ*-bisabolene	5.03	ND	6.12	2.48	ND	1516	
	t*rans*-*γ*-bisabolene	ND	ND	0.60	ND	ND	1533	
	γ-bisabolene	ND	ND	ND	ND	22.30	1529	
	germacrene-*B*	ND	1.30	ND	ND	ND	1561	

*VBH: Vismia baccifera Hechicera; VBJ: Vismia baccifera Jají; VBV: Vismia baccifera El Valle; VBM: Vismia baccifera La Mucuy; VMT: Vismia macrophylla Táchira. The composition of the essential oil was determined by comparison of the MS of each component with Wiley GC/MS library data and also from its retention index (RI). Percentage ratio (% PR): Number of compounds/ Total compounds *100. ND: not detected*

Table 1. Chemical composition of essential oils of two *Vismia* species collected in different locations in Venezuela Andes (*Continued)*

Type sesquiterpene	Compounds	VBH	VBJ	VBV	VBM	VMT	RI*	%PR
Bicyclic sesquiterpenes	*cis*-cadina-1(6),4-diene	ND	ND	ND	ND	0.20	1383	VBH= (18/ 9; 50%) VBJ= (15/ 8; 53.33%) VBV= (17/ 6; 35.29%) VBM= (16/ 7;43.75%) VMT= (30/ 15; 50%)
	7-epi-sesquithujene	ND	ND	1.10	ND	ND	1403	
	trans-caryophyllene	ND	ND	ND	ND	5.30	1413	
	β-caryophyllene	2.58	45.06	7.35	18.47	ND	1415	
	cis-*α*-bergamotene	0.96	ND	ND	ND	ND	1422	
	trans-*α*-bergamotene	3.82	ND	3.28	0.74	2.10	1434	
	α-guaiene	2.62	ND	0.68	0.74	1.10	1438	
	3,7-guaiadiene	1.09	ND	ND	ND	ND	1436	
	5,8-daucadiene	ND	ND	ND	ND	0.50	1456	
	epi-bicyclosesquiphellandrene	ND	ND	ND	ND	0.30	1458	
	6,9-guaiadiene	ND	1.80	ND	ND	ND	1442	
	selina-4,11-diene	ND	ND	ND	3.95	ND	1475	
	trans-1,4-cadinadiene	ND	ND	ND	ND	1.30	1469	

Type sesquiterpene	Compounds	VBH	VBJ	VBV	VBM	VMT	RI*	%PR
	γ-muurolene	ND	ND	ND	ND	6.10	1474	
	α-amorphene	1.91	3.67	ND	ND	ND	1472	
	β-selinene	ND	1.74	0.49	8.82	1.90	1484	
	trans-4,5-muuroladiene	ND	ND	ND	ND	0.80	1488	
	α-selinene	1.29	3.14	ND	11.63	4.90	1494	
	α-muurolene	ND	ND	ND	ND	3.50	1497	
	α-bulnesene	1.31	ND	ND	ND	ND	1502	
	β-himachalene	ND	2.87	ND	ND	ND	1506	
	γ-cadinene	ND	1.73	ND	ND	4.90	1510	
	α-cadinene	ND	ND	ND	ND	1.00	1534	
	δ-cadinene	1.57	4.91	0.66	ND	10.7	1527	
	7-epi-*α*-selinene	ND	ND	ND	0.64	ND	1522	
Tricyclic sesquiterpenes	*α*-cubebene	1.36	1.73	0.50	2.27	0.60	1347	VBH= (18/ 2; 11.11%) VBJ= (15/ 2; 13.33%) VBV= (17/ 2; 11.76%) VBM= (16/ 2; 12.50%) VMT= (30/ 4; 13.33%)
	α-ylangene	ND	ND	ND	ND	0.60	1367	
	α-copaene	2.40	3.66	0.93	4.71	2,00	1374	
	β-copaene	ND	ND	ND	ND	1.00	1420	
Monocyclic sesquiterpenes oxygenateds	*β*-bisabolol	ND	ND	ND	ND	4.40	1665	VBH, VBJ, VBV, VBM= 0% VMT= (30/ 2; 6.67%)
	α-bisabolol	ND	ND	ND	ND	0.70	1681	
Bicyclic sesquiterpenes oxygenateds	epi-*α*-cadinol	ND	ND	ND	ND	0.40	1636	VBH, VBJ, VBV y VBM= 0%; VMT= (30/ 2; 6.67%)
	epi-*α*-muurolol	ND	ND	ND	ND	1.70	1646	

*VBH: Vismia baccifera Hechicera; VBJ: Vismia baccifera Jají; VBV: Vismia baccifera El Valle; VBM: Vismia baccifera La Mucuy; VMT: Vismia macrophylla Táchira. The composition of the essential oil was determined by comparison of the MS of each component with Wiley GC/MS library data and also from its retention index (RI). Percentage ratio (% PR): Number of compounds/ Total compounds *100. ND: not detected*

The previous investigations carried out with same species collected in other locations in Merida state showed that essential oil of *Vismia baccifera* Triana and Planch collected from Chiguara at 900 m.a.s.l. was mainly composed by germacrene-*D* (15.8%), *α*-cadinol (14.5%), epi-*α*-cadinol (11.9%), *β*-caryophyllene (10.1%) and *δ*-cadinene, while same species collected in La Hechicera at 1989 m.a.s.l. showed *β*-caryophyllene (45.7%), valencene (12.3%), *β*-elemene (10.7%), *α*-humulene (8.9%) and germacene-*D* (6.3%) as major components [25]. Furthermore, Vizcaya et.al. [32]; studied the volatile compounds obtained from the bark of *Vismia baccifera* var. *dealbata* Triana and Planch collected from Chiguara. Analysis carried out by GC-MS revealed the presence of 13 components being caryophyllene oxide (31.4%), *β*-caryophyllene (26.4%) and *α*-zingiberene (12.6%) the main components.

In addition, the essential oil from *Vismia macrophylla* Kunth leaves collected from Michelena, Táchira State showed *β*-caryophyllene (20.1%), germacrene-*D* (11.6%) and *β*-elemene (7.0%) as main components [24], while the essential oil from the fruits of same species was mainly composed by germacrene-*D* (12.1%), *δ*-cadinene (10.7%) and *γ*-bisabolene (22.3%) [21].

Another investigation conducted with the essential oil of *V*. *guianenesis* (Aubl.) Choisy fruits collected form Pernambucano-Brasil revealed thought GC-MS analysis the presence of 38 components, mainly sesquiterpenes, where *β*-caryophyllene (25.8%), *α*-copaene (13.1%) and *δ*-cadinene (11.6%) were observed as main components. [22]. Likewise, *V. guianensis* leaves collected from Itacoatiara, Brazil showed (E)-caryophyllene (10.40%), *α*-copaene (29.45%), and (E)-nerolidol (24.06%) while *V. cayennensis* leaves was mainly composed by germacrone (25.42%) and curzerene (25.29%) [33].

Table 2. Antimicrobial activity of essential oils of *Vismia baccifera* y *Vismia macrophylla*. Fruits

Microorganisms	Essential oils (mm)					Inhibition zone (mm)							MIC (µL/ mL)				
						Antibiotics											
	VBH	VBJ	VBV	VBM	VM	LI	VA	CE	AZ	PI	FI	VR	VBH	VBJ	VBV	VBM	VM
S. aureus (ATCC 25923	16	15	25	20	14	46	NT	NT	NT	NT	NT	NT	80	150	100	100	150
E. faecalis (ATCC 29212)	15	19	NA	23	14	NT	22	NT	NT	NT	NT	NT	80	60	NT	200	250
E. coli (ATCC 25922)	8	NA	11	NA	8	NT	NT	36	NT	NT	NT	NT	920	NT	520	NT	740
K. pneumoniae (ATCC 23357)	13*	7*	13*	NA	NA	NT	NT	NT	46	NT	NT	NT	550	920	580	NT	NT
P. aeruginosa (ATCC 27853)	13*	NA	12*	15*	NA	NT	NT	NT	NT	26	NT	NT	700	NT	620	400	NT
C. albicans (CDC-B385)	10*	11*	11*	10*	NA	NT	NT	NT	NT	NT	36	NT	700	350	800	400	NT
C. krusei (ATCC 6258)	19*	8*	15*	15*	NA	NT	NT	NT	NT	NT	NT	28	300	920	400	400	NT

Inhibition zone (discs 6 mm of diameter); VBH: Vismia baccifera Hechicera; VBJ: Vismia baccifera Jají; VBV: Vismia baccifera El Valle; VBM: Vismia baccifera La Mucuy; VMT: Vismia macrophylla Táchira NA: not active, NT: not tested; LI: Linezolid® (30 µg/ Oxoid™); VA: Vancomicyn® (30 µg/ Liofilchem®); CE: Cefuroxime® (30 µg/ Oxoid™); AZ: Aztreonam® (30 µg/ BD BBL™), PI: Piperacilin® (100 µg/ Oxoid™); FI: Fluconazole ® (100 µg / Oxoid™); VR: Voriconazole (400 µg/ Oxoid™); MIC: Minimum inhibitory concentration range between 50 to 950 µL/mL

Antimicrobial activity was also performed on the oils under investigation through the disk diffusion method described by Velasco et al (2007) [29]. All samples analyzed showed (Table 2), growth inhibition against *S. aureus* with MIC values between 80 to 150 µL/mL. *E*. *faecalis* was also inhibited by VBJ (60 µL/mL); VBH (80 µL/mL); VBM (200 µL/mL); VM (250 µL/mL). VM, VBM and VBV showed a rather light activity against *E*. *coli* with MIC values between 520 µg/mL to 980 µg/mL. *K*. *pneumoniae* was inhibited by VBH, VBV and VBJ with MIC values of 550 µL/mL, 580 µL/mL and 980 µL/mL, respectively. Oils from samples VBM, VBV and VBH also showed activity against the Gram-negative bacteria *P*. *aeruginosa* with MIC values between 400 µL/mL to 700 µL/mL. Furthermore, oils from *V*. *baccifera* proved to be active against *C*. *albicans* and *C*. *krusei* with MIC values between 300 µL/mL to 950 µL/mL.

According to the results, both *Vismia* species revealed a wide range of antimicrobial activity since the oils were able to inhibit the growth not only of Gram-positive and Gram-negative bacterial strains but two yeast strains as well. Previous investigations carried out with *Vismia baccifera* var *dealbata* collected from Chiguará, Mérida state, Venezuela showed that oil extracted from the barks of this species has strong activity against *Candida krusei* (1.6 µg/mL), moderate activity against *Candida glabrata* (200 µg/mL) and low activity against *Candida tropicalis*, *Candida parapsilosis* and *Cryptococcus neoformans* with MIC values of 1000 µg/mL each [32]. “In addition, the essential oil from the fruits of the same species but collected form La Hechicera, Mérida State, Venezuela, showed a broad spectrum of antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with MIC values ranging from 9 to 37 µg/mL” [23].

“Furthermore, oil obtained from the fruits of *V*. *macrophylla* showed antibacterial activity against Gram-positive (*S*. *aureus* and *E*. *faecalis*) as well as Gram-negative bacteria (*E*. *coli*), with MIC values ranging from 150 µL/mL to 740 µL/mL while the oil obtained from leaves were active against *S*. *aureus* (100 µL/mL) and *E*. *faecalis* (500 µL/mL) and also showed activity against *Candida albicans* and *C*. *krusei* (600 µL/mL, each)” [21].

Another investigation carried out with the fruits of *Vismia guianensis* from Pernambucano-Brasil showed activity against *Bacillus cereus* and *Staphylococcus aureus* at the concentration of 10 µL/mL, while for *Staphylococcus epidermidis*, *Staphylococcus lentus* and *Vibrio alginolyticus* the MIC value was 100 µL/mL [22]. Likewise, a study of *V. guianensis* and *V. cayenensis* (Jacq.) Pers. leaves collected from Itacoatiara, Brazil showed activity against *V. guianenesis* essential oil against the yeast *C. parapsilosis* with MIC value of 1.56 µg/mL, whereas essential oil of *V. cayenensis* was active against *E. coli* (50 µg/mL) and *S. aureus* (25 µg/mL) as well as the yeast *C. parapsilosis* (50 µg/mL) [33].

It is important to mention that bacteriostatic activity of essential oils might be related to the kind of components present in the sample. It has been documented that terpenes, especially mono and sesquiterpenes have the capacity to inhibit the bacterial growth through several mechanisms such as: cell membrane denaturation, nutrient transport interference, metabolic regulation changes, electrolyte exchange disruption (K^+/H^+), among others [34]. Moreover, it should be noted that Gram-positive bacteria exhibit a cellular wall composed mainly of peptidoglycans which might be more susceptible to the essential oil diffusion while Gram-negative bacteria cellular wall present a bilayer of lipoproteins that confers more resistance to the effect caused by the chemical components presents in essential oils [21].

5. CONCLUSIONS

Essential oils of two *Vismia* species collected at different locations in Venezuelan Andean were analyzed through GC and GC/MS techniques. Results showed that oils were composed mainly of sesquiterpenes being *α*-curcumene, *β*-curcumene, *β*-caryophyllene, germacrene D, *γ*-bisabolene and *α*-selinene among the major components. Antimicrobial assays were also performed in samples under investigation. A broad spectrum of activity was observed since all samples analyzed showed growth inhibition against *S. aureus* and *E. faecalis* with MIC values between 60 to 250 µL/mL. Gram-negative bacteria *P. aeruginosa* was inhibited by the species *V. baccifera* with MIC values between 400 µL/mL to 700 µL/mL. In addition, oils from *V. baccifera* also showed activity against *C. albicans* and *C. krusei* with MIC values between 300 µL/mL to 950 µL/mL. Results indicate that *Vismia* species might be considered as an alternative to aid infectious diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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