Original Research Article

Chemical composition and antibacterial activity of essential oils from fruits of two *Vismia* species collected at different locations in Venezuelan Andes

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 by terpenes, anthrones, lignans, flavonoids, anthraquinones, steroids and xanthones. The purpose of present 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 collectedat 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 were carried out by disc diffusion assays.**Results**: GC and GC/MS analysis showed that these species are mainly composed by 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 (*Candidaalbicans and Candidakrusei*) as well. **Conclusions**: According to the results observed in present investigation, *Vismia* species might be considered as an alternative to aid infectious diseases.

Keywords: *Vismiamacrophylla*, *Vismiabaccifera*, 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 (Figure 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 *Vismiabaccifera*Triana& Planch and *Vismiamacrophylla*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, prenylatedanthrones, lignans, sterols, flavonoids, flavonols, anthraones, anthraquinones, bianthraquinones, benzophenones, steroids and xanthones, among the most common isolated and characterized components (Figure 2) [4, 8 ,11].

Regarding the pharmacological activities, a number of 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, eczema, among others [4,9-12].

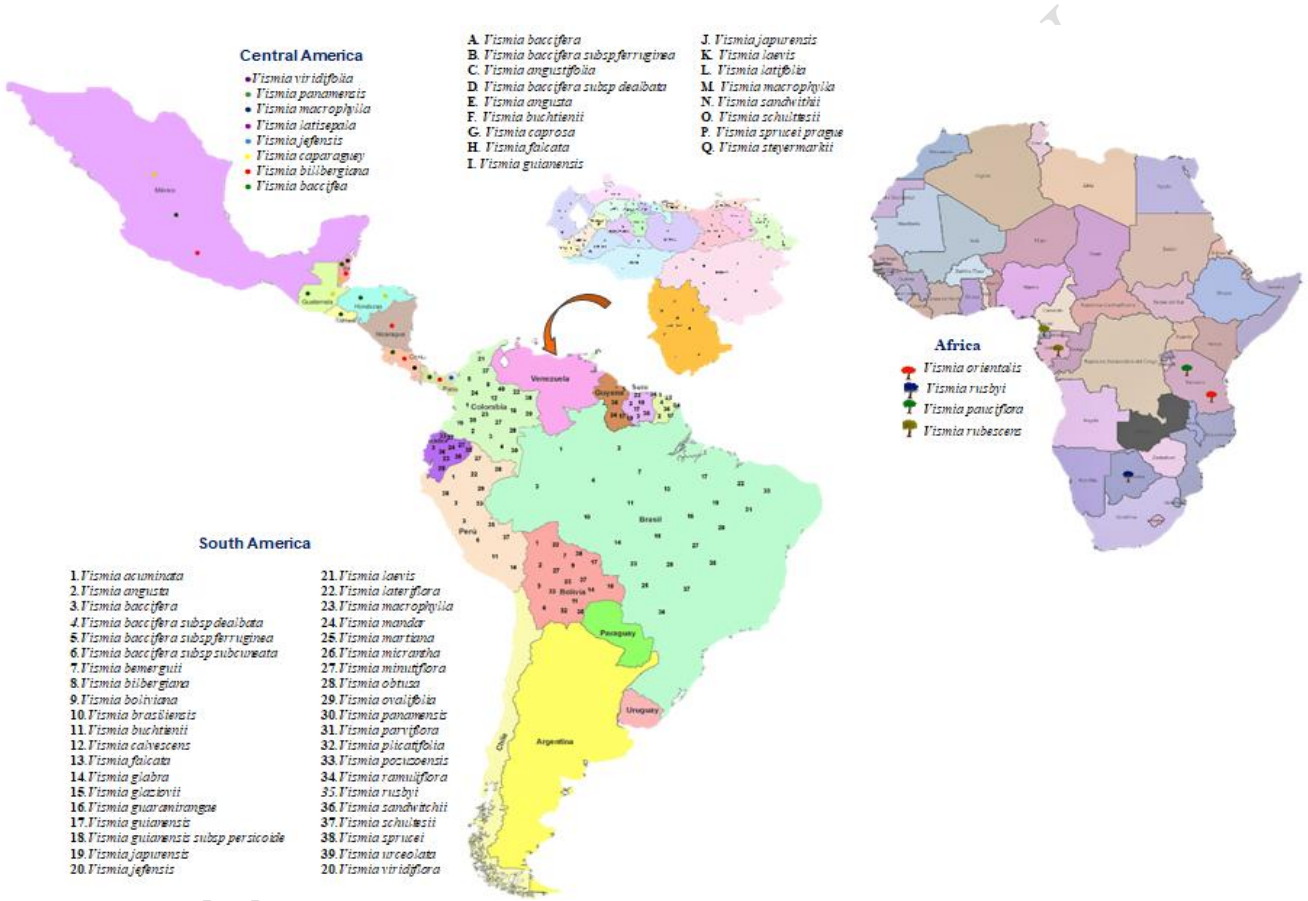

Figure 1. Distribution of *Vismia* genus in Central America, South America and Africa

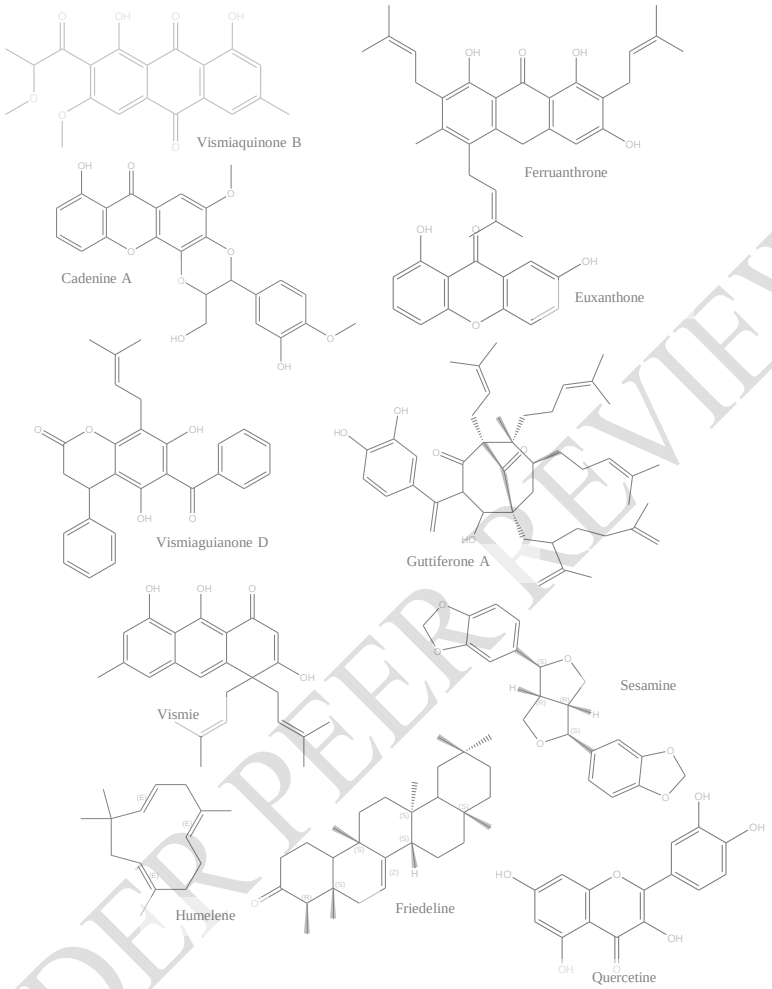

Figure 2. Secondary metabolites most commonly isolated from different species of *Vismia* genus

*Vismiamacrophylla*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, *Vismiabaccifera* (L.) Planch. & Triana, a species typically found at 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 ~~by~~ ofterpen-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 *Vismiamacrophylla* 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 *Staphylococcusaureus*, *Enterococcusfaecalis*, *Escherichiacoli*, *Klebsiellapneumoniae* and *Pseudomonasaeruginosa*[23].

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.

2. Materials and Methods

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 (Figure 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 1 835 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**.

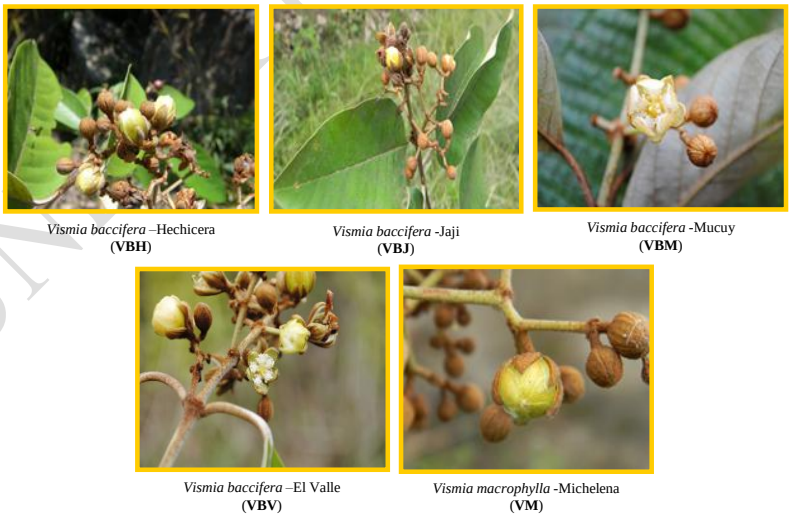

Figure 3. *Vismiabacciera* and *Vismia macrophylla* species collected at different locations in Venezuela Andean

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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.

Comment [R1]: Explain why different weights and different weights by volumes were used, especially if you did a comparative study of their efficacy.

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% phenylmethylpolysiloxane 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 [26,27].

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 (6^{th} Ed.) and NIST 05 data base library, followed by comparisons of mass spectral (**MS**) data with published literature [26] and the retention index calculation.

Bacterial Strains: the microorganisms and yeast used for the antimicrobial method were *Staphylococcusaureus* (ATCC 25923), *Enterococcusfaecalis* (ATCC 29212), *Escherichiacoli* (ATCC 25992), *Klebsiellapneumoniae* (ATCC 23357), *Pseudomonasaeruginosa* (ATCC 27853), *Candidaalbicans* (CDCB 385) and *Candidakrusei* (ATCC 6258).

Comment [R2]: Yeast is a microorganism as well

Antimicrobial Method: The antimicrobial activity was carried out according to the disc diffusion assay described by [28]. 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.

Antifungal activity was also evaluated following the disc diffusion methodology described by National Committee for Clinical Laboratory Standards [29]. 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). 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 at least 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 [30]. 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 at least twice.

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 will be performed by using the Duncan´s multiple ranges test. Significance level has been established at $\alpha = 0.10$.

Comment [R3]: Indicate the exact number of replicates

Comment [R4]: Were these serial dilutions?

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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 (Figure 4).differences between main components of samples analyzed: **VBH** was mainly composed ~~by~~ of 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%);V**BM**β-curcumene (18.3%) and β-caryophyllene (18.5%);**VM** showed γ-bisabolene (22.3%) and α-selinene (11.6%) as major components.

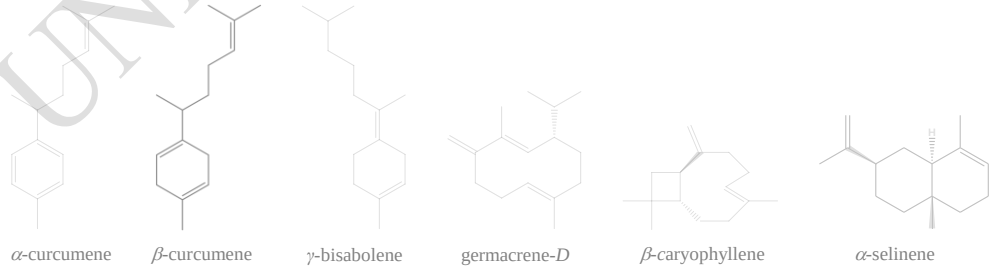

Fig. 4. *Major components identified in V. baccifera and V. macrophylla in present investigation.*

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).

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

VBH: *Vismiabaccifera* Hechicera; **VBJ**: *Vismiabacciferа*Jají; **VBV**: *Vismiabaccifera* El Valle; **VBM**: *Vismiabaccifera* La Mucuy; **VMT**: *Vismiamacrophylla*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

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					0.20	1383	
	7-epi-sesquithujene			1.10			1403	
	trans-caryophyllene					5.30	1413	**VBH=** (18/ 9;50%)
	β-caryophyllene	2.58	45.06	7.35	18.47		1415	**VBJ=** (15/ 8;53.33%)
	cis-*α*-bergamotene	0.96					1422	**VBV=** (17/ 6; 35.29%)
	trans-*α*-bergamotene	3.82		3.28	0.74	2.10	1434	**VBM=** (16/ 7;43.75%)
	α-guaiene	2.62		0.68	0.74	1.10	1438	**VMT=** (30/ 15; 50%)
	3,7-guaiadiene	1.09					1436	
	5,8-daucadiene					0.50	1456	

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	epi-bicyclosesquiphellandrene					0.30	1458	
	6,9-guaiadiene		1.80				1442	
	selina-4,11-diene				3.95		1475	
	trans-1,4-cadinadiene					1.30	1469	
	γ-muurolene					6.10	1474	
	α-amorphene	1.91	3.67				1472	
	β-selinene		1.74	0.49	8.82	1.90	1484	
	trans-4,5-muuroladiene					0.80	1488	
	α-selinene	1.29	3.14		11.63	4.90	1494	
	α-muurolene					3.50	1497	
	α-bulnesene	1.31					1502	
	β-himachalene		2.87				1506	
	γ-cadinene		1.73			4.90	1510	
	α-cadinene					1.00	1534	
	δ-cadinene	1.57	4.91	0.66		10.7	1527	
	7-epi-*α*-selinene				0.64		1522	
Tricyclic sesquiterpenes	*α*-cubebene	1.36	1.73	0.50	2.27	0.60	1347	**VBH=** (18/ 2;11.11%)
	α-ylangene					0.60	1367	**VBJ=** (15/ 2;13.33%)
	α-copaene	2.40	3.66	0.93	4.71	2,00	1374	**VBV=** (17/ 2;11.76%)
	β-copaene					1.00	1420	**VBM=** (16/ 2;12.50%)
								VMT= (30/ 4;13.33%)
Monocyclic sesquiterpenes oxygenateds	*β*-bisabolol					4.40	1665	**VBH, VBJ, VBV, VBM=** 0%
	α-bisabolol					0.70	1681	**VMT=** (30/ 2;6.67%)
Bicyclic sesquiterpenes oxygenateds	epi-*α*-cadinol					0.40	1636	**VBH, VBJ, VBV** y **VBM=** 0%;
	epi-*α*-muurolol					1.70	1646	**VMT=**(30/ 2;6.67%)

VBH: *Vismiabaccifera* Hechicera; **VBJ**: *Vismiabaccifera*Jají; **VBV**: *Vismiabaccifera* El Valle; **VBM**: *Vismiabaccifera* La Mucuy; **VMT**: *Vismiamacrophylla*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

Previous investigations carried out with same species collected in other locations in Merida state showed that essential oil of *Vismiabaccifera*Triana and Planch collected from Chiguara at 900 m.a.s.l. was mainly composed ~~by~~ of 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*., 2014 [31]; studied the volatile compounds obtained from the bark of *Vismiabaccifera* 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 *Vismiamacrophylla*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~~ ofgermacrene-*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~~ ofgermacrone (25.42%) and curzerene (25.29%)[32].

Antimicrobial activity was also performed on the oils under investigation through the disk diffusion method described by Velasco et al (2007) [28]. 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 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 yeasts strains as well. Previous investigations carried out with *Vismiabaccifera*var*dealbata* collected from Chiguará, Mérida state, Venezuela showed that oil extracted from the barks of this species has strong activity against *Candidakrusei* (1.6 µg/mL), moderate activity against *Candidaglabrata* (200 µg/mL) and low activity against *Candidatropicalis*, *Candidaparapsilosis* and *Cryptococcusneoformans* with **MIC** values of 1000 µg/mL each [31].In addition, the essential oil from the fruits of same species but collected form La Hechicera, Mérida State, Venezuela, showed a broad spectrum of antibacterial activity against *Staphylococcusaureus*, *Enterococcusfaecalis*, *Escherichiacoli*, *Pseudomonasaeruginosa* and *Klebsiellapneumoniae* 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 *Candidaalbicans* and *C. krusei* (600 µL/mL, each) [21].

Another investigation carried out with the fruts of *Vismiaguianensis* from Pernambucano-Brasil showed activity against *Bacilluscereus* and *Staphylococcusaureus* at the concentration of 10 µL/mL, while for *Staphylococcusepidermidis*, *Staphylococcuslentus* and *Vibrioalginolyticus* 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 activityagainst*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) [32].

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 [33]. Moreover, it should be noted that Gram-positive bacteria exhibit a cellular wall composed mainly by 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 present in essential oils [21].

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

Comment [R9]: Indicate ND rather than shading

Microorganisms	Essential oils (mm)					Inhibition zone (mm) Antibiotics							MIC (µL/ mL)				
	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**							80	150	100	100	150
***E. faecalis* (ATCC 29212)**	15	19	NA	23	14		**22**						80	60	**NT**	200	250
***E. coli* (ATCC 25922)**	8	**NA**	11	**NA**	8			**36**					920	**NT**	520	**NT**	740
***K. pneumoniae* (ATCC 23357)**	13*	7*	13*	**NA**	**NA**				**46**				550	920	580	**NT**	**NT**
***P. aeruginosa* (ATCC 27853)**	13*	**NA**	12*	15*	**NA**					**26**			700	**NT**	620	400	**NT**
***C. albicans* (CDC-B385)**	10*	11*	11*	10*	**NA**						**36**		700	350	800	400	**NT**
***C. krusei* (ATCC 6258)**	19*	8*	15*	15*	**NA**							**28**	300	920	400	400	**NT**

Inhibition zone (discs 6 mm of diameter); **VBH**: *Vismiabaccifera*Hechicera; **VBJ**: *Vismiabaccifera*Jají; **VBV**: *Vismiabaccifera* El Valle; **VBM**: *Vismiabaccifera* La Mucuy; **VMT**: *Vismiamacrophylla*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.

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 ~~by~~ ofsesquiterpenes 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 in management of (or control of) infectious diseases.

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Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Ethics Approval and Consent to Participate

Not applicable.