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Full Length Research Paper

Chemical composition, antibacterial and antioxidant activities of the essential oil from *Vismia guianensis* fruits

Raquel G. Silvestre[1], Marcilio M de Moraes[1], Antonio C. S. Lins[2], Maria T. Ralph[3], José V. Lima-Filho[3], Celso A. Camara[1] and Tania M. S. Silva[1]*

[1]Departamento de Ciencias Moleculares, Universidade Federal Rural de Pernambuco, Recife-Pernambuco, CEP 52171-900, Brazil.
[2]Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, João Pessoa-Paraiba, CEP 58051-970, Brazil.
[3]Departamento de Biologia, Universidade Federal Rural de Pernambuco, Recife-Pernambuco, CEP 52171-900, Brasil.

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In recent decades, the essential oils of plants have drawn great interest as sources of natural products. Essential oil from the fruits of *Vismia guianensis* was tested for its chemical constituents and antimicrobial and antioxidant activities. Gas chromatography/mass spectrometry (GC-MS) analysis of the essential oil revealed the presence of 38 sesquiterpenoids. The major components were β-caryophyllene (25.8%), α-copaene (13.1%), and δ-cadinene (11.6%). Antimicrobial activities were measured against six species of Gram negative and seven species of Gram positive bacteria and showed antibacterial activity against the human pathogenic Gram-positive bacteria *Staphylococcus lentus* with minimum inhibitory concentration (MIC) values of 78 µg/ml. The antioxidant activity of the essential oil was evaluated using the beta carotene/linoleic acid assay and showed antioxidant activity.

Key words: *Vismia guianensis*, chemical composition, antibacterial, antioxidant, fruits, essential oil.

INTRODUCTION

Clusiaceae (Guttiferae) is a family almost exclusively tropical in distribution and comprises about 43 genera and 1610 species. In Brazil, there are 21 genera and about 180 species with wide occurrence (Barroso et al., 2002). Extensive phytochemical studies have shown Clusiaceae to be a rich source of secondary metabolites including xanthones, triterpenoids, flavonoids, lactones and organic acids. In addition, plants of this family produce a series of oxidized and polyisoprenylated benzophenones, some of which are structurally complex and biologically active (Cuesta-Rubio et al., 2005), as well as essential oil components that are more hydrophobic in nature (Crockett, 2010). *Vismia guianensis* (Aulb.) Choisy (Clusiaceae) is a tree native to tropical America, and occurs in Brazil, Colombia, Guyana and Venezuela (Ewan, 1962). The tree is found as colonizers of man-made clearings and natural gaps in forests and in abandoned or active agricultural areas in Brazilian Amazonia (Albuquerque, 1980); is commonly known in Brazil as “lacre”, and the stem bark is used in popular medicine as a laxative and in the treatment of various types of dermatitis (Correa, 1926). Quinoids (Gonzales et al., 1980; Delle Monache et al., 1980; Botta et al., 1986), xanthones (Botta et al., 1986), prenylated benzo-phenones, and benzocoumarins have been reported previously from *V. guianensis* (Seo et al., 2000). To the best of our knowledge, the antimicrobial and antioxidant activities of the essential oils from *V. guianensis* fruits have not been previously studied. The aim of this study was to analyze the chemical composition of the essential oil from *V. guianensis* fruits and evaluate its antimicrobial and antioxidant activities; this work is a continuation of the investigations into the pharmacologically active

*Corresponding author. E-mail: taniasarmento@dcm.ufrpe.br. Tel: +558133206382.

compounds from this species (Lins et al., 2007).

MATERIALS AND METHODS

The fruits of *V. guianensis* were collected in Cabo de Santo Agostinho, Pernambuco in March 2011. The voucher specimen of this collection (49.815) was deposited at the Herbarium Professor Vasconcelos Sobrinho, Universidade Federal Rural de Pernambuco.

Isolation of the essential oil

The fresh fruits (2.0 kg) of *V. guianensis* were submitted to hydrodistillation in a Clevenger-type apparatus at 100°C for 4 h. The distilled oil was extracted with hexane and dried over anhydrous sodium sulfate. After filtration, the yield of the essential oil was 0.63 (0.03%, w/w). The oil was then preserved in a sealed dark glass vial at 5°C until required.

Analysis of essential oil

The composition of the essential oil from *V. guianensis* was determined by the use of analytical GC flame ionization detector (FID) and GC/MS techniques. The same column and analysis conditions were used for both GC and GC/MS. A Hewlett-Packard 5890 Series II GC apparatus equipped with a J&W Scientific DB-5MS fused silica capillary column (30 cm × 0.25 mm i.d.), a split-splitless injector heated at 240°C and a FID at 240°C was used. The oven temperature was programmed as follows: initial temperature of 60°C for 1.0 min, and increase 3°C/min up to 240°C. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The injection volume was 1.5 µl (split ratio 1:30). GC/MS analyses were performed using a Varian 220-MS IT GC system with a mass selective detector, mass spectrometer in EI 70 eV with a scan interval of 0.5 s and fragments from 40 to 550 Da. The components were identified by comparison of the mass spectra with the NIST21 and NIST107 mass spectral libraries of the GC-MS data system, and by comparison of their RI with the relevant literature data (Adams, 2007). The relative amount (RA) of each individual component in the essential oil was expressed as the percentage of the peak area relative to the total peak area. The retention indices (RI) value of each component was determined relative to the retention times (RT) of a series of C_7-C_{37} *n*-alkanes with linear interpolation on the column.

Antioxidant activity in linoleic acid oxidation

This experiment was carried out using the method from Emmons et al. (1999) with some modifications. β-Carotene (10 mg) was dissolved in 1 ml of chloroform, and 50 µl was added to 530 µl of linoleic acid and 40 µl of Tween 20. Oxygenated deionized water (100 ml) was added and mixed well. Aliquots of 3 ml of the carotene/linoleic acid emulsion were mixed with a sample of the essential oil (100 and 50 µg /ml) and incubated in a water bath at 50°C. Oxidation of the emulsion was monitored spectrometrically by measuring the absorbance at 470 nm over a 120 min period. The control sample contained solvent in the place of the essential oil. Antioxidant activity was expressed as percentage of inhibition relative to the control from 20 to 120 min incubation period using the following equation:

$$AA=100(DR_C-DR_S)/DR$$

Where, AA is the antioxidant activity, DR_C is the degradation rate in the presence of the control (=Abs_i-Abs_f), DR_S is the degradation rate in the presence of the sample (=Abs_i-Abs_f), Abs_i is the initial absorbance at time 0, and Ab_f is the absorbance at 20, 40, 60, 80, 100, and 120 min. Trolox (a water-soluble Vitamin E analog) at 16 µg/ml was used as the reference antioxidant.

Antibacterial activity

Agar-well diffusion method

The antibacterial activity of the oil was screened through the agar-well diffusion method (Perez et al., 1990). The following bacterial species were used in the assays: (Gram-negative) *Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Vibrio vulnificus, V. alginolyticus, V. parahaemolyticus, Salmonella enteric* Ser. Typhimurium; and (Gram-positive) *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Staphylococcus xylosus, Staphylococcus epidermidis* and *S. lentus*. The bacteria were cultured in Mueller Hinton agar (10^8 cells/ml; 0.5 of the MacFarland standard), and the wells (5 mm in diameter) were filled with 20 µl of essential oil at concentrations of 100, 50, 25 and 12.5 mg/ml (w/v) diluted in dimethyl sulfoxide (DMSO). DMSO alone was used as the control. The results were expressed as the mean±SD of the growth inhibition zone in millimeters. All assays were performed in duplicate.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC concentration of the oil was determined using the broth dilution method (Koneman et al., 2001). In this case, only susceptible bacterium where the dosage of 100 mg/ ml produced inhibition zones larger than 10 mm in the agar-well diffusion method was tested. The MIC was the lowest oil concentration between 10 to 0.009 mg/ml that caused visible inhibition of growth. From these tubes, an aliquot of 0.1 ml was added to the Petri dishes containing Mueller Hinton agar to determine the MBC defined as the lowest concentration resulting in no growth after the incubation period time of 24 h at 37°C. All assays were performed in duplicate.

Statistical analysis

All samples were analyzed in triplicate unless stated otherwise, and the results were expressed as the mean±SD. All statistical analyses were carried out using the Microsoft Excel software package (Microsoft Corp., Redmond, WA). Differences between the inhibition zones (mean±SD) produced by distinct dosages of the EO were compared by Student's *t test* or ANOVA with $P<0.05$.

RESULTS AND DISCUSSION

Chemical analysis

The essential oil was obtained in 0.03% yield by hydrodistillation of the *V. guianensis* fruits. Quantitative analyses of the chemical composition are shown in Table 1. GC-MS analysis revealed the presence of 38 sesquiterpenoids from 99.2% of the compounds identified. Chemical identification of the oil constituents was conducted based on RT, RI, mass spectral data, and by an internet search of mass spectral databases. The

Table 1. Chemical composition of the essential oil from the fruits of *V. guianensis*.

Compound[a]	RI[b]	RA (%)[c]
γ-Elemene	1330	0.2±0.0
α-Cubebene	1340	0.7±0.0
α-Ylangene	1370	0.7±0.0
α-Copaene	1378	13.1±0.8
β-Elemene	1390	2.7±0.0
Cyperene	1403	0.1±0.0
β-Caryophyllene	1421	25.8±0.6
β-Copaene	1427	1.4±0.1
α-Guaiene	1434	0.5±0.1
Aromadendrene	1441	0.8±0.0
6,9-Guaiadiene	1444	0.2±0.0
cis-Muurola-3,5-diene	1450	1.0±0.1
α-Humulene	1453	4.7±0.2
allo-Aromadendrene	1458	0.6±0.0
cis-Cadina-1,4-diene	1461	0.2±0.0
4,5-Di-epi-aristolochene	1469	0.1±0.0
β-Chamigrene	1477	3.1±0.0
γ-Muurolene	1479	3.9±0.4
Germacrene D	1487	1.8±0.3
Aristolochene	1490	0.3±0.0
β-Selinene	1492	7.8±0.3
δ-Selinene	1494	8.8±0.3
cis-*β*-Guaiene	1495	1.3±0.0
δ-Amorphene	1513	2.3±0.1
γ-Cadinene	1516	11.6±0.9
δ-Cadinene	1524	0.5±0.0
trans-Cadina-1,4-diene	1533	0.9±0.0
α-Cadinene	1537	0.7±0.0
Selina-3,7(11)-diene	1545	0.3±0.0
cis-Muurol-5-en-4β-ol	1550	0.6±0.1
epi-longipinanol	1560	0.3±0.3
Longicamphenylone	1563	0.2±0.1
Viridiflorol	1590	0.2±0.1
Guaiol	1599	0.4±0.0
epi-1,10-di-cubenol	1620	0.1±0.0
epi-α-Cadinol	1638	0.4±0.1
Cubenol	1644	0.4±0.1
Pogostol	1650	0.6±0.2
Sesquiterpenes		93.3±1.2
Sesquiterpenes Oxygenated		5.8±0.7
Not identified		0.9±0.0
Identified		99.2±0.5
Total		100%

[a] The identified constituents are listed in their order of elution; [b] RI, retention indices calculated against C_7-C_{37} *n*-alkanes on the DB-5 column; [c] RA , relative amount (peak area relative to the total peak area).

chemical structures of the three principal identified compounds are shown in Figure 1. All compounds were sesquiterpenes, of which only 5.8% were oxygenated sesquiterpenes. The three main sesquiterpenes identified were β-caryophyllene (25.8%), α-copaene (13.1%), and δ-cadinene (11.6%). In the literature, only two other

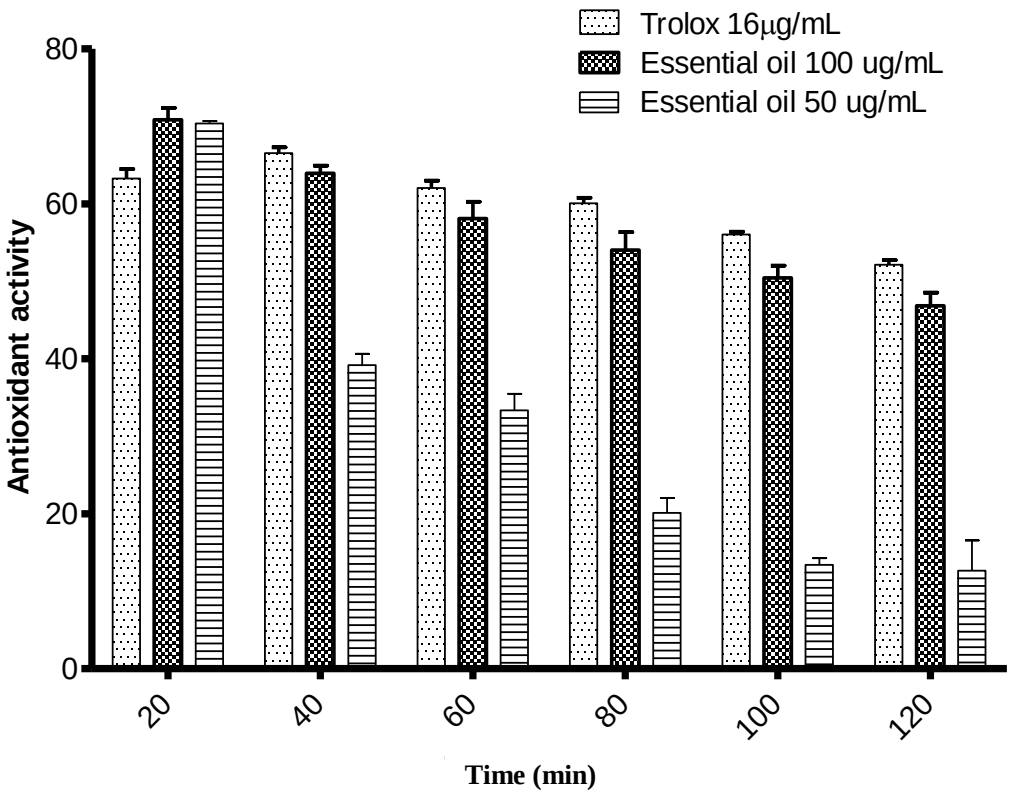

Figure 1. Antioxidant activities of the fruits from *V. guianensis* using the carotene bleaching method.

species of the *Vismia* genus were studied for essential oil compositions; the fruits from *Vismia baccifera* Triana & Planch. and *Vismia macrophylla* Kunth leaves showed differences in the composition of essential oils. Rojas et al. (2011) reported that *Vismia baccifera* contained three main sesquiterpenes: *trans*-cadin-1,4-diene (36.6%), *cis*-cadin-1,4-diene (18.8%) and β-caryophyllene (11.9%).

In the essential oil of *Vismia macrophylla* leaves, the major components were β-caryophyllene (20.1%), germacrene D (11.6%) and β-elemene (7.0%). The essential oil from leaves of *V. baccifera* var. dealbata collected from two different locations in Venezuela showed several differences in both collections. Germacrene-D (15.8%), α-cadinol (14.5%), *epi*-α-cadinol (11.9%), β-caryophyllene (10.1%) and δ-cadinene (7.5%) were the major constituents in one sample while β-caryophyllene (45.7%), valencene (12.3%), β-elemene (10.7%), α-humulene (8.9%), and germacrene-D (6.3%) were major components in another sample (Buitrago et al., 2009). When our results were compared to those in literature, the compositions of the essential oils showed similarities. All the *Vismia* species showed sesquiterpenes as main components.

Antioxidant activity

The β-carotene bleaching assay is based on the loss of the yellow color of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed in the presence of antioxidants (Oke et al., 2009). Polyunsaturated fatty acids, such as linoleic acid, are easily oxidized by oxygen in the air. This auto-oxidation leads to the occurrence of chain reactions with the formation of coupled double bonds. At a later stage, auto-oxidation also leads to the presence of secondary products such as aldehydes, ketones, and alcohols. During the application of the β-carotene/linoleic acid method, the oil showed linoleic acid inhibition activity (Figure 1).

Antibacterial activity

Antibacterial activity of essential oil from the fruits of *V. guianensis* is shown in Table 2. The oil showed a selective range of antibacterial activity against Gram-positive bacteria such as Staphylococcal species. However, the MIC values were likely higher than 1000 μg/ml, except for *Staphylococcus lentus*, which was specifically susceptible at a concentration of 78 μg/ml. This bacterium is an opportunistic human pathogen that is also involved with goat mastitis (Karachalios et al., 2006). It has frequently been reported that Gram-positive bacteria are more susceptible to essential oils than Gram

Table 2. Antimicrobial activities of the essential oil of *V. guianensis* fruits.

Strain	Growth inhibition zones in millimeters (mean±SD)				MIC	MBC
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml		
Gram-negative						
Klebsiella pneumoniae	-	-	-	-	NT	NT
Pseudomonas aeruginosa	-	-	-	-	NT	NT
Escherichia coli	-	-	-	-	NT	NT
Vibrio vulnificus	-	-	-	-	NT	NT
Vibrio alginolyticus	8.0±1.0	7.0±0.5	-	-	NT	NT
Vibrio parahaemolyticus	-	-	-	-	NT	NT
Salmonella enterica Ser. Typhimurium	-	-	-	-	NT	NT
Gram-positive					NT	NT
Listeria monocytogenes	-	-	-	-	NT	NT
Bacillus cereus	10.5±0.5	9.0±0.5	8.0±0.5	8.0±1.0	> 10	
Staphylococcus aureus	17.0±3.0	10.0±0.5	9.0±0.5	8.5±0.5	> 10	
Staphylococcus xylosus	-	-	-	-	NT	NT
Staphylococcus epidermidis	8.5±0.5	7.0±0.5	-	-	NT	NT
Staphylococcus lentus	10.0±1.0	8.0±0.5	-	-	0.078	-

NT, not tested; MIC, minimal inhibitory; MBC, minimum bactericidal concentration.

(-) bacteria (Burt, 2004; Kalemba and Kunicka, 2003), which is supported by our findings. Although the antibacterial activity of plant extracts from *Vismia* species has been reported previously (Nguemeving et al., 2006; Tamokou et al., 2009), similar results with essential oil from *V. guianensis* fruits were not previously described. Rojas et al. (2011) reported a broad range of antibacterial activity from the oil of *V. baccifera* against several human pathogens. In this case, the major components present in the oil were *trans*-cadin-1,4-diene (36.6%), *cis*-cadin-1,4-diene (18.8%) and β-caryophyllene (11.9%). Antimicrobial compounds from the terpenoid class have been commonly described in plant essential oils (Botelho et al., 2007), but a synergy among several chemical compounds is also feasible (Sonboli et al., 2006).

In this study, the major compound in oil from *V. guianensis* was the sesquiterpene β-caryophyllene (25.8%), which has been described by its antibacterial activity (Dorman and Deans, 2000). Although the spectrum of antimicrobial activity from the oil of *V. guianensis* was narrow, it represents a prospective source of specific antibacterial compounds.

Conclusion

Essential oils possess antimicrobial and antioxidant characteristics, as well as several biological activities. In our study, the oil from *V. guianensis* fruits showed antioxidant activity. While this oil exhibited apparent selective antibacterial activity against Gram-positive human pathogens, their use as a prospective source of antimicrobial agents could be of interest for the pharmacological industry.

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