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Insecticidal activity of three species of *Guatteria* (Annonaceae) against *Aedes aegypti* (Diptera: Culicidae)

Actividad insecticida de tres especies de *Guatteria* (Annonaceae) contra *Aedes aegypti* (Diptera: Culicidae)

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Abstract: The products of vegetal origin were assessed for bioactive substances to reduce reliance on organophosphate and pyrethroid insecticides, to which insect populations have become resistant. For this reason the aim of this study was to assess whether the essential oils of *Guatteria hispida, G. blepharophylla* and *G. friesiana* have insecticidal effect against *A. aegypti* under laboratory conditions. Essential oils were extracted through hydrodistillation using a modified Clevenger apparatus and analyzed by Gas Chromatography (CG-FID), Gas Chromatography coupled to Mass Spectrometry (GC-MS), and Nuclear Magnetic Resonance (NMR). The bioassays were analyzed according to the *Probit* model. The GC-MS and NMR analyses confirmed that the leaves of *G. blepharophylla* have the caryophyllene oxide as their main component; in *G. friesiana* the α-,β- and γ-eudesmols prevail, and in *G. hispida* α- and β-pinene, and (*E*)-caryophyllene are the predominant compounds. The lethal concentrations LC_{50}, LC_{95} and LC_{99}, were respectively 85.74, 199.35 and 282.76ppm for *G. hispida;* 58.72, 107.6 and 138.37ppm for *G. blepharophylla;* and 52.6, 94.37 and 120.22ppm for *G. friesiana.* The oil extracted from *G. friesiana* presented the best insecticidal effect.

Key words: Mosquito control. Dengue. Essential oils. Larvicides

Resumen: Se evalúan productos de origen vegetal en busca de sustancias bioactivas que tengan la capacidad de reducir la dependencia de insecticidas organofosforados y piretroides, a los que las poblaciones de insectos se han vuelto resistentes. Por esta razón el objetivo de este estudio fue evaluar si los aceites esenciales de *Guatteria hispida, G. blepharophylla* y *G. friesiana* presentan efecto insecticida contra *A. aegypti* bajo condiciones de laboratorio. Los aceites esenciales se extrajeron a través de hidrodestilación por medio de un aparato tipo Clevenger, analizados por Cromatografía Gaseosa acoplada a Espectrometría de Masas (CG-EM) y Resonancia Magnética Nuclear (RMN). Los bioensayos se analizaron de acuerdo con el modelo Probit. Los análisis de (CG-EM) y (RMN) confirmaron que las hojas de *G. blepharophylla* presentan óxido de cariofileno como el principal componente; en *G. friesiana* fue α-, β- y γ-eudesmol, y en *G. hispida* α- y β-pineno y (*E*)-cariofileno fueron los compuestos predominantes. La concentraciones letales CL_{50}, CL_{95} y CL_{99} fueron respectivamente 85,74, 199,35 y 282,76ppm para *G. hispida*; 58.72, 107.6 y 138.37 ppm para *G. blepharophylla;* 52,6, 94,37 y 120,22ppm para *G. friesiana.* El aceite extraído de *G. friesiana* presentó el mejor efecto insecticida.

Palabras clave: Control de mosquitos. Dengue. Aceites esenciales. Larvicidas.

Introduction

Dengue cases and their clinical complications appear in countries of tropical and subtropical regions every year, with no promising prospects for future decrease of this problem (Guzmán *et al.* 2006). Unplanned urbanization, demographic and climatic changes in conjunction with the fast human migrations worldwide through air and land transport facilities are increasing the spread of the dengue arboviruses and its vector, *Aedes aegypti* (Linnaeus, 1762) to new places (Kroeger and Nathan 2007).

In the absence of a vaccine that confers permanent immunity to the four serotypes of the DENV1-4 dengue and their genetic variations, vector control is used as the key measure to fight the disease (Hombach 2007; Periago and Guzmán 2007). However, even with the extensive accumulated knowledge over decades on this problem and knowing that so far that the only viable possibility is the direct vector control; this is not efficient since the epidemics in countries of tropical and subtropical regions continue occurring.

The dependence on synthetic organophosphorus (OP) and pyrethroid (P) insecticides to combat both immature and adult forms of the vector mosquito has been the most frequently adopted procedure for years, despite its little impact on the reduction of dengue cases. Unfortunately, such procedure has favored the outburst of *A. aegypti* populations

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that are insecticide tolerant at concentrations that otherwise would cause mortality to susceptible individuals (WHO 1992). This phenomenon has been reported in several regions such as: Venezuela (Mazzarri and Georghiou 1995), the Caribbean (Rawlins 1998), Singapore (Ping *et al.* 2001), Cuba (Rodriguez *et al.* 2002), Peru (Chávez *et al.* 2005), Thailand (Ponlawat *et al.* 2005), Argentina, Bolivia (Biber *et al.* 2006), México (Flores *et al.* 2006) and Brazil (Montella *et al.* 2007).

Botanical-origin products emerge as a promising alternative to control the vector of the dengue virus, after being set aside between the 30's and the 50's because the discovery of chemical synthetic insecticides (organochlorines, organophosphates, carbamates and pyrethroids). Besides have proven insecticidal effect, the plant-based products display a diversity of compounds with attractive, dislodging or repellent features that could be used in integrated pest management systems, as alternatives aimed at monitoring and control the mosquito populations (Isman 2006; Navarro-Silva *et al.* 2009).

The Annonaceae family comprises approximately 130 genera with 2.300 species of tropical and subtropical distribution (Kessler 1993). This group of plants has well known economic importance due to the trade of its fruits, byproducts, pharmacological activity, raw material for cosmetics, perfume industry, natural medicine and antimicrobial and insecticidal activity compounds (Costa *et al.* 2008, 2009; Boyom *et al.* 2003; Isman 2006). The genus *Guatteria* Ruiz & Pav, belongs to this family, with approximately 290 species distributed throughout Mesoamerica, the Caribbean and South America (Erkens and Maas 2008) with no study related to its insecticidal activity yet. The history of biological potential of the Annonaceae and its antimicrobial activity described in Costa *et al.* (2008), related with the chemical composition of essential oils of three species of the genus *Guatteria*, *Guatteria blepharophylla* (Mart.), *Guatteria friesiana* (W.A. Rodrigues) and *Guatteria hispida* (R.E. Fries) (Erkens and Maas 2008), raised the hypothesis of its insecticidal properties.

Research has been carried out to determine the potential efficacy of derivates from plants in vector control programs. The environmentally safe and biodegradable botanical insecticides could be an alternative method of control, owing to the growing incidence of the insect resistance to synthetic insecticides. In view of the abovementioned and the records of the insecticide action of the Annonaceae Family and the antimicrobial activity of the genus *Guatteria*, the essential oils of these species were evaluated for their effect against *A. aegypti* larvae under laboratory conditions.

Materials and Methods

Sample Collection. With the purpose to observe some variance in the chemical constituents on the essential oils of the species of *Guatteria* (*G. blepharophylla, G. friesiana,* and *G. hispida*) the collection was made in the same months, as well as, of the same species used by Costa *et al.* (2008), but three years later. Leaves of *G. blepharophylla* were collected in January 2008, on the campus of the Federal University of Amazonas (UFAM); Leaves of *G. friesiana* were collected in January 2008, at the Experimental Farm of the Federal University of Amazonas (UFAM), and leaves of *G. hispida* were collected in July 2008, at the Adolpho Ducke Forest Reserve. Voucher specimens were deposited in the Herbarium of the Department of Biology, UFAM, Manaus, AM, Brazil, under registration numbers 7340, 7341 and 7707, respectively. Leaves were obtained from flowered plants.

Extraction of essential oils. The leaves (250g) of the three *Guatteria* species were randomly collected dried at room temperature for three days, grounded and subjected to hydrodistillation for 4 hours, using a modified Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate (Na_2SO_4) and the percentage content was calculated based on the dry weight. The extraction was repeated three times.

Gas Chromatography (GC-FID) analysis. The GC analyses were carried out using a Shimadzu GC-17A fitted with a flame ionization detector (FID) and an electronic integrator. Separation of the compounds was achieved employing a ZB-5MS fused capillary column (30m x 0.25mm x 0.25µm film thickness) coated with 5%-phenyl-arylene-95%-methylpolysiloxane. Conditions of injection were performed according to Costa *et al.* (2008): injector temperature 240ºC; oven temperature program of 60ºC-300ºC at a rate of 3ºC/min; split 20:1 during 1.50 min, carrier gas He: 1 mL/min, constant flow; sample volume 0.5 µL.

Gas Chromatography - Mass Spectrometry GC-MS analysis. The GC-MS analyses were performed on a Shimadzu QP5050A GC/MS system equipped with an AOC-20i autoinjector. A J&W Scientific DB-5MS (coated with 5%-phenyl-95%-methylpolysiloxane) fused capillary column (30m x 0.25mm x 0.25µm film thickness) was used as the stationary phase. The conditions of injection were the same as described above and according to Costa *et al.* (2008). The mass spectrometer was operated at 70eV. The constituents of the essential oils were identified by comparison of their mass spectral pattern and retention indices (RI) with those given in the literature (Adams 2007). The retention indices (RI) were calculated according to Van Den Dool and Kratz (1963).

1D/2D ^{1}H and ^{13}C Nuclear Magnetic Resonance analysis (NMR). The crude essential oils of these species were analyzed by Nuclear Magnetic Resonance (NMR) of ^{1}H and ^{13}C 1D/2D. Nuclear Magnetic Resonance (NMR) spectra were recorded in a Bruker Avance 400 spectrometer operating at 9.4 Tesla, observing ^{1}H at 400 MHz and ^{13}C at 100 MHz. Chloroform was used as the deuterated solvent. Chemical shifts values were given in parts per million (ppm) relative to the tetramethylsilane (TMS), used as internal reference standard (δ 0.00).

Determination of insecticidal activity. The larvae from the Rockefeller Colony - CDC (Center of Disease Control) were kept in plastic trays (35.5cm x 21.5cm x 6.5cm) containing 3.000mL of dechlorinated water under controlled temperature (25ºC±1), humidity (70%±10) and photoperiod (12:12) conditions in a climatized chamber Model 347 CDG, at the Laboratory of Medical and Veterinary Entomology. Thus they remained there until reaching the stage of final 3rd instar and initial 4th instar, a change observed from the exuviate. The latter did not receive any food or chemical treatment.

After reaching the larval stage described above, the larvae were counted, separated and transferred with a Pasteur pipette to disposable plastic glasses with a 50mL capacity,

containing 20mL of the same dechlorinated water, in a total of 10 larvae per glass. Then, these larvae were exposed to different concentrations of essential oils from the *Guatteria* spp. (12, 15, 20, 35, 40, 50, 60, 65, 80, 85, 95 and 120 ppm). The amount of oil for each concentration was placed in plastic containers of 330mL capacity containing Dimethyl sulfoxide (DMSO) 1% (BIOTEC® brand, with 99% purity) and 80mL of mineral water, in a final volume of 100mL.

All the experiments were repeated four times, including a control treatment exclusively with DMSO and mineral water. Finally, seven concentrations were tested (12, 15, 20, 35, 40, 60 and 85ppm).

Mortality of the larvae exposed to the treatment was determined after 24 hours, considering mortality within a confidence interval of 95%. Larvae unable to reach water surface when touched were considered dead (WHO 1981a, 1981b). In parallel, the DMSO calibration was achieved in five concentrations between 1% and 5% to confirm that the 1% percentage used in the assays in fact did not cause mortality of the larvae. Data of the bioassay and solvent calibration were subjected to the *Probit* analysis (Finney 1971; Raymond 1985).

Results and Discussion

The yields of essential oils were 0.6% for *G. friesiana*, 0.5 for *G. hispida*, and 0.3 for *G. blepharophylla*. These results of the GC-FID and GC-MS analyses are similar to those obtained by Costa *et al.* (2008) (Table 1). The analyses of ^{1}H and ^{13}C 1D/2D NMR spectral data of the crude essential oils of *G. friesiana* and *G. blepharophylla*, along with the analysis of these crude essential oils by GC-FID and GC-MS (Table 1), confirm the results reported by Costa *et al.* (2008) that *G. friesiana* are dominated by α-eudesmol (15.1%), β-eudesmol (52.0%) and γ-eudesmol (24.0%) (Fig. 1A-C), respectively, while *G. blepharophylla* is dominated by caryophyllene oxide (70.0%) (Fig. 1D). From the actual ^{1}H and ^{13}C NMR spectral along with GC-FID and GC-MS (Table 1) analyses of the crude essential oil of *G. hispida*, the three major compounds, α-pinene (31.0%), β-pinene (36.0%) and (*E*)-caryophyllene (21.0) (Fig. 1E-G respectively), were confirmed after extensive analysis by Barero *et al.* (1995); Lee (2002); Hall *et al.* (2005), and suggested by the GC-MS performed by Costa *et al.* (2008). The ^{1}H and ^{13}C NMR data

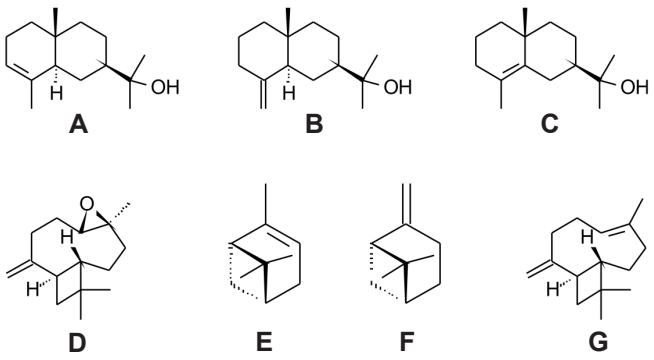

Figure 1. Major components identified in the essential oils of *Guatteria* leaves. **A**: α-eudesmol; **B**: β-eudesmol; **C**: γ-eudesmol; **D**: caryophyllene oxide; **E**: α-pinene; **F**: β-pinene; and **G**: (*E*)-caryophyllene.

obtained for compounds α-, β-, γ-eudesmols, caryophyllene oxide, α-, β-pinenes, and (*E*)-caryophyllene in the crude essential oils were in accordance with Barero *et al.* (1995); Lee (2002); Hall *et al.* (2005) and Costa *et al.* (2008).

Mortality tests with the *A. aegypti* larvae under laboratory conditions using essential oils of the three *Guatteria* species indicated a strong larvicida action, with the highest rates being recorded for $LC_{50,95}$ and $_{99}$ for *G. hispida,* and the lowest rates for *G. friesiana* (Table 2). By comparing the confidence intervals (CI) of each lethal concentration (LC) of the three species, it becomes evident that *G. hispida* is not in the same category as of the others. However, *G. blepharophylla* and *G. friesiana* were similar according to IC. The same behavior of insecticide action was observed in relation to the Angular Coefficient (AC) of concentrations and mortality response for the three species. Finally, all the experiments were significantly adjusted to the Probit ($p < 0.05$) model ($x^2 = 0.318$, 3.20 and 3.58, Table 2).

Considering the increase of *A. aegypti* insecticide-resistant populations, mosquito control requires candidates to replace organophosphates (OP) and pyrethroids (P) chemical pesticides with products of proven insecticide effectiveness while these are environmentally safe. New plant records with biological action emerge every day, standing as possible substitutes to be incorporated into the fight against Culicidae.

Table 1. Essential oil composition of *Guatteria* species.

Constituents			Leaf oil %		
	RI[a]	RI[b]	*Guatteria blepharophylla*	*Guatteria friesiana*	*Guatteria hispida*
α-Pinene (**1E**)	931	932			31.0
Camphene	944	946			0.4
β-Pinene (1F)	977	974			36.0
Myrcene	988	988			0.5
p-Cymene	1022	1020			0.1
Limonene	1028	1024			0.4
Citronellal	1150	1148	0.1		
α-Terpinen-4-ol	1172	1174			1.0
α-Terpineol	1188	1186			0.1
γ-Elemene	1333	1335	0.1		

(Continúa)

(Continuation Table 1).

Constituents	RI[a]	RI[b]	Leaf oil %		
			Guatteria blepharophylla	*Guatteria friesiana*	*Guatteria hispida*
Cyclosativene	1368	1369	0.1		
α-Ylangene	1373	1373	0.9		0.3
β-Bourbonene	1388	1387	0.2		
β-Elemene	1390	1389	1.2		
(*E*)-Caryophyllene (**1G**)	1419	1417	1.0		21.0
β-Ylangene	1420	1419	0.1		
α-*Trans*-bergamotene	1434	1432	0.3		0.1
Aromadendrene	1437	1439	0.1		
α-Humulene	1451	1452			0.7
(*E*)-β-Farnesene	1456	1454			0.5
Trans-cadina-1-(6),4-diene	1475	1475			0.2
γ-Gurjunene	1477	1475	0.2		0.6
γ-muurolene	1478	1478	0.2		
γ-curcumene	1482	1481	0.7		
γ-Himachalene	1483	1481			1.4
Germacrene D	1487	1484	0.1		
e*pi*-Cubebol	1492	1493	0.1		
β-Selinene	1493	1489	0.4		0.8
Cuparene	1500	1504			
β-Bisabolene	1507	1505	0.3		
γ-Cadinene	1511	1513	0.2		
δ-Cadinene	1523	1522	1.1		1.2
Trans-cadina-1(2),4-diene	1530	1531c	0.4		
α-Calacorene	1540	1544	0.7		
Elemol	1551	1548		2.2	
Trans-cadinene-ether	1554	1557	2.0		
Germacrene B	1554	1559	0.3		
Spathulenol	1578	1577		2.5	
Caryophyllene oxide (**1D**)	1582	1582	70.0		3.0
β-Copaen-4-α-ol	1592	1590	0.7		
Humulene epoxide II	1609	1608	2.2		0.1
10-epi-γ-Eudesmol	1620	1622		1.3	
1-*epi*-Cubenol	1627	1627	0.3		
Cis-cadin-4-en-7-ol	1632	1635	1.6		
γ-Eudesmol (**1C**)	1632	1630		24.0	
Caryophylla-4(14),8(15)-dien-5-ol	1635	1636c	0.6		
Hinesol	1641	1640		0.9	
Cubenol	1643	1645	0.4		
β-Eudesmol (**1B**)	1652	1649		52.0	
α-Eudesmol (**1A**)	1656	1652		15.1	
14-hydroxy-9-epi-(*E*)-Caryophyllene	1670	1668	2.5		
Ishwarone	1679	1680	2.0		
14-oxy-α-muurolene	1770	1767	0.2		
Carissone	1926	1926		0.2	
Monoterpenes identified			**0.1**		**68.4**
Sesquiterpenes identified			**91.2**	**98.2**	**31.0**
Total identified			**91.3**	**98.2**	**99.4**

RI[a] (calc.), retention indices on ZB-5MS column calculated according to Van Den Dool and Kratz (1963). RI[b] retention indices according to Adams, 2007. [c]Costa *et al.* (2008).

Table 2. Values of lethal concentrations (LC) that cause mortality in 50, 95 and 99 % of the *A. aegypti* larvae and confidence intervals (IC)

Lethal concentrations	*Guatteria hispida*	*Guatteria blepharophylla*	*Guatteria friesiana*
LC_{50}	85.74 ppm	58.72 ppm	52.60 ppm
IC	(74.05 – 112.78)	(55.08 – 62.81)	(50.11 – 55.17)
LC_{95}	199.35 ppm	107.64 ppm	94.37 ppm
IC	(140.19 – 435,37)	(95.28 – 128.16)	(85.96 – 107.09)
LC_{99}	282.76 ppm	138.37 ppm	120.22 ppm
IC	(108.92 – 769.05)	(117.98 – 174.53)	(106.09 – 142.82)
x^2	0.318	3.20	3.58
Slope	4.48 ± 0.89	6.25 ± 0.62	6.48 ± 0.55

There was no mortality in the control groups. LC_{50} lethal concentration that causes death in 50% of larvae exposed, LC_{95} lethal concentration that caused the death of 90% and LC_{99} lethal concentration that causes death in 99% of larvae exposed, slope ± standard deviation, X^2 chi-square (Finney 1971).

The extraction yield of vegetable essential oils is a factor to consider in botanic products with biological action. When compared to the yield of oils extracted from other Annonaceae published in literature (Boyom *et al.* 2003), it becomes evident that the *Guatteria* species analyzed produced higher yields, especially considering that only 250g of leaves from each species were used for the entire extraction process. The type of compound and its chemical characteristics are fundamental factors to determine whether an extract or essential oil can act as an insecticide. According to Costa *et al.* (2008), the essential oils of the three *Guatteria* species are formed by mono and sesquiterpenes. According to NMR data the major compounds in *G. friesiana* are β-eudesmol, γ-eudesmol, and α-eudesmol (Fig. 1A-C respectively); in *G. blepharophylla,* caryophyllene oxide (Fig. 1D), and in *G. hispida* α-pinene, β-pinene, and (*E*)-caryophyllene (Fig. 1E-G respectively), agreeing with the results of Costa *et al.* (2008). Determining the relation between the insecticide activity and the chemical composition of the essential oils is a significant challenge, because the possibility of synergic activities is always present, which makes difficult to establish an efficient model for this purpose. However, contrasting analyses of oil composition, its major compounds and the abundance of its constituents are often carried out in an attempt to assign the mortality action observed.

The results achieved in this study lead to the hypothesis that sesquiterpene-rich essential oils can be considered more active in the control of *A. aegypti* in relation to those containing more monoterpenes. This conclusion becomes evident by comparing activity results of *G. friesiana, G. blepharophylla* against *G. hispida.* The first two are significantly richer in sesquiterpenes than the latter, which contains monoterpenes (Costa *et al.* 2008). The same pattern can be observed in the study developed by Santos *et al.* (2006) with *Cordia leucomalloides* (Jack) (LC_{50}=63.1 ppm) and *C. curassavica* (Jack) (Boraginaceae) (LC_{50}=97.7), active in the *A. aegypti* larvae control, the first species being both more active and richer in sesquiterpenes; however, these are less effective than the plants analyzed here. Similarly, Simas *et al.* (2004) indicate that the best insecticidal activity was detected for the isolated (*E*)-nerolidol (LC_{50}=17 ppm), sesquiterpenes of *Myroxylon balsamun* (L.) Harms (Fabaceae). Despite that its LCs is lower than that found in the present study, it reinforces the idea that the sesquiterpenes are highly effective when compared with monoterpenes.

Although studies developed by Santos *et al.* (2006) and Simas *et al.* (2004) evidenced a stronger action of the sesquiterpenes, this pattern was not found in Costa *et al.* (2005) with oils of *Hyptis martiussi* Benth. (Lamiaceae), *Lippia sidoides* Cham. (Verbenaceae) and *Syzigium aromaticum* (L.) Merr. & Perry (Myrtaceae), whose major compounds are the monoterpenes (1,8-cineol, timol and eugenol), presenting LCs_{50} similar to these reported by Santos *et al.* (2006) and Simas *et al.* (2004) against *A. aegypti* larvae.

The comparison between LC_{50} and LC_{95} indicates that diterpenes could have an even more toxic effect than sesquiterpenes for this mosquito species, as shown with *Copaifera reticulata* Ducke (Fabaceae) for diterpenoid acid 1[(-)-3β-acetoxylabdan-8(17)-13-dien-15-oic] (LC_{50}=0.8ppm and LC_{95}=8.2ppm) and acid 2{alepterolic [(-)-3β-hydroxylabdan-8(17)-13-dien-15-oic]} (LC_{50} = 87.3ppm and LC_{95} =128.8ppm) (Geris *et al*. 2008). More interesting is the fact that tetranortriterpenoids (azadirachtin) have presented less effect than monoterpenes, sesquiterpenes and triterpene according to the LCs for *A. aegypti* (Wandscheer *et al.* 2004). By observing the results in Furtado *et al.* (2005) and Cavalcanti *et al.* (2004) (Table 3), only the essential oils of a few plants present the LCs with values close to the determined by *G. friesiana*, and *G. blepharophylla* which places the essential oils of these species among the most active against *A. aegypti* larvae. Finally, we recommend conducting studies to reveal the mechanisms of action of the major compounds of these oils in the mosquito.

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Table 3. Comparison between lethal concentrations (LCs) of the essential oils from several plant species evaluated as larvicidal against *A. aegypti* with their major compounds, respectively.

Vegetal species	Chemical compound	LC_{50}	LC_{90}	LC_{95}
Vanillosmopsis arborea Baker (Compositae)[a]	α-bisabolol	15.9 mg/mL	28.5 mg/mL	-
Lippia sidoides Cham (Verbenaceae)[a]	Timol	45.4 mg/mL	48.2 mg/mL	-
Cymbopogon winterianus Jowitt (Graminaceae)[a]	Citronelal	54.6 mg/mL	88.2 mg/mL	-
Ageratum conyzoides L (Asteraceae)[a]	Precocene	61.5 mg/mL	95.1 mg/mL	-
Cymbopogon citratus Stapf (Graminaceae)[a]	Neral	63.8 mg/mL	112.2 mg/mL	-
Ocimum basilicum purpurascens Benth (Labiadae)[a]	Linalool	66.9 mg/mL	88.3 mg/mL	-
Ocimum tenuiflorum L (Labiadae)[a]	Eugenol	71.2 mg/mL	111.6 mg/mL	-
Tagetes minuta L (Asteraceae)[a]	Diidrotagetone	72.8 mg/mL	104.8 mg/mL	-
Citrus limon L (Rutaceae)[a]	Limonene	95.8 mg/mL	102.8 mg/mL	-
Ocimum gratissimun L (Labiadae)[a]	Eugenol	104.5 mg/mL	195.1 mg/mL	-
Ocimum gratissimum L (Labiadae)[b]	Eugenol	60 ppm	-	-
Lippia sidoides Cham (Verbenaceae)[b]	Timol	63 ppm	-	-
Ocimum americanum L (Labiadae)b	*E*-Methyl-Cinnamate	67 ppm	-	-
Cymbopogon citratus Stapf (Gramineae)[b]	Geranial	69 ppm	-	-
Hyptis suaveolens Poit (Labiadae)[b]	1,8-Cineole	261 ppm	-	-
Alpinia zerumbet (Pers.) Burtt & Smith (Zingiberaceae)[b]	1,8-Cineole	313 ppm	-	-
Syzygium jambolana DC (Myrtaceae)[b]	Z-Ocimene	433 ppm	-	-
Citrus limonia Osbeck (Rutaceae)[b]	Limonene	519 ppm	-	-
Citrus sinensis Osbeck (Rutaceae)[b]	Limonene	538 ppm	-	-
Guatteria hispida (Fries) Erkens & Maas Annonaceae)[c]	α- and β-pinenes and (*E*)-caryophyllene	85.7 ppm	-	199.3 ppm
G. blepharophylla (Mart) Mart (Annonaceae)[c]	Caryophyllene oxide	58.7 ppm	-	107.6 ppm
G. friesiana (Rodrigues) Erkens & Maas (Annonaceae)[c]	α-, β- and γ-eudesmols	52.6 ppm	-	120.2 ppm

[a] Furtado *et al*., (2005); [b] Cavalcanti *et al.,* (2004) and [c] our result; - Data are not comparable.

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