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Chemical composition and antimicrobial activity of the essential oils of the Amazon *Guatteriopsis* species

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ABSTRACT

The essential oils of *Guatteriopsis blepharophylla*, *Guatteriopsis friesiana* and *Guatteriopsis hispida* were obtained by hydrodistillation and analysed by GC and GC/MS. The main compound found in the leaf oil of *G. blepharophylla* was caryophyllene oxide (**1**) (69.25%). The leaf oil of *G. friesiana* contained predominantly β-eudesmol (**2**) (51.60%), γ-eudesmol (**3**) (23.70%), and α-eudesmol (**4**) (14.56%). The major constituents identified in the leaf of *G. hispida* were β-pinene (38.18%), α-pinene (30.77%) and (*E*)-caryophyllene (20.59%). The antimicrobial activity of the essential oils was evaluated against 11 species of microorganisms. The oil of *G. friesiana* exhibited significant antimicrobial activity for all microorganisms tested, whereas that of *G. hispida* and *G. blepharophyla* had potent activity against *Rhodococcus equi* with MIC of 50 μg mL^{-1}. The major constituents of each oil were also tested separately, and showed lower activity compared to the oils. Moreover, mixtures of the main constituents, in the same proportions found in *G. friesiana* and *G. hispida* oils, did not show the same activity as the original oils.

1. Introduction

The Annonaceae is a large family of tropical and subtropical trees and shrubs comprising about 120 genera and more than 2000 species (Leboeuf et al., 1982). Economically, it is has appreciable importance as a source of edible fruits, raw material for the cosmetics and perfume industries, and as medicinal plants (Leboeuf et al., 1982; Boyom et al., 2003; Braga, 1976; Gemtchújnicov, 1976). Despite the importance of the members of this family in folk medicine, the number of species that have been chemically investigated is still small (*ca* 150 species belonging to 41 genera) (Castedo et al., 1991). The main chemical constituents in this family are alkaloids, carbohydrates, lipids, aminoacids, proteins, polyphenols, terpenes, aromatic compounds and acetogenins (Leboeuf et al., 1982; Rupprecht et al., 1990). Although the Annonaceae have a wide diversity of chemical constituents, most studies have focused on their alkaloidal contents, which are of chemotaxonomic relevance (Castedo et al., 1991). *Guatteriopsis* is a small genus in this family known as "envireira", comprising about four species of small trees, of which the majority is distributed throughout Central and South America (Hutchinson, 1964). Only one phytochemical study on this genus has been reported, which describes the isolation and identification of essential oils from the leaf of *Guatteriopsis blepharophylla* (Maia et al., 2005). However, there is nothing reported involving the biological activities of either the compounds or the extracts from this genus.

In the present paper we report the chemical compositions and antimicrobial activities of the essential oils from leaves of *G. blepharophylla*, *Guatteriopsis friesiana* and *Guatteriopsis hispida*, three species of Amazon Brazilian plants.

The plant species were selected based on the fact that many species of this family are used in traditional medicine for various purposes (Boyom et al., 2003; Braga, 1976; Gemtchújnicov, 1976) and on the absence of more detailed phytochemical (except *G. blepharophylla*) and biological studies.

The three species present themselves as a small tree known as "envireira", with a thick yellowish bark, found mainly in Brazilian Amazon (Ribeiro et al., 1999) having no popular uses described in the academic literature.

2. Results and discussion

The leaf essential oils were obtained from the plants with 0.24%, 0.50% and 0.41% yield (relative to dried material weight) respectively for *G. blepharophylla*, *G. friesiana* and *G. hispida*. The results

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of the analysis of the essential oils are given in Table 1. The oils contain mainly monoterpenes and sesquiterpenes. Sesquiterpenes represent more than 90% of the oils of *G. blepharophylla* (90.56%) and *G. friesiana* (97.13%), and 29.25% of *G. hispida*. Monoterpenes represent more than 70% of the oil of *G. hispida*. The oils displayed some qualitative and quantitative differences (see Table 1), although the volatile constituents have been previously reported in other species of the family Annonaceae. The main constituent found in the leaf oil of *G. blepharophylla* was caryophyllene oxide (**1**) (69.25%). The leaf oil of *G. friesiana* contained predominantly β-eudesmol (**2**) (51.60%), γ-eudesmol (**3**) (23.70%) and α-eudesmol (**4**) (14.56%) (Fig. 1). The major constituents identified in the leaf oil of *G. hispida* were β-pinene (38.18%), α-pinene (30.77%) and (*E*)-caryophyllene (20.59%). The eudesmol isomers were successfully separated by preparative TLC using a doped silica gel with $AgNO_3$. All the isomers were further characterized by NMR (Nuclear Magnetic Resonance) spectroscopic analyses.

Table 1
Chemical composition of the essential oils of *Guatteriopsis* species

Constituents	RI[a]	*G. hispida* (%)	*G. blepharophylla* (%)	*G. friesiana* (%)
α-Pinene	934	30.77		
Camphene	944	0.43		
β-Pinene	977	38.18		
Myrcene	991	0.47		
p-Cymene	1022	0.10		
Limonene	1026	0.36		
Citronellal	1152		0.08	
α-Terpinen-4-ol	1174	0.08		
α-Terpineol	1188	0.13		
δ-Elemene	1336		0.11	
Cyclosativene	1368		0.12	
α-Ylangene	1373	0.27	0.89	
β-Bourbonene	1388		0.21	
β-Elemene	1390		1.18	
(*E*)-caryophyllene	1420	20.59	0.99	
β-Ylangene	1425		0.12	
α-*Trans*-bergamotene	1434	0.13	0.33	
Aromadendrene	1441		0.10	
α-Humulene	1451	0.70		
(*E*)-β-Farnesene	1458	0.46		
Trans-cadina-1-(6),4-diene	1475	0.16		
γ-Gurjunene	1479	0.63	0.24	
γ-muurolene	1478		0.15	
γ-curcumene	1482		0.68	
γ-Himachalene	1485	1.44		
Germacrene D	1489		0.14	
epi-Cubebol	1492		0.14	
β-Selinene	1493	0.78	0.39	
Cuparene	1496			
β-Bisabolene	1507		0.31	
γ-Cadinene	1511		0.18	
δ-Cadinene	1523	1.20	1.07	
Trans-cadina-1(2),4-diene	1531		0.38	
α-Calacorene	1541		0.69	
Elemol	1551			2.16
Trans-cadinene-ether	1551		2.03	
Germacrene B	1554		0.31	
Spathulenol	1578			2.64
Caryophyllene oxide (1)	1581	2.77	69.25	
β-Copaen-4-α-ol	1592		0.70	
Humulene epoxide II	1609	0.12	1.98	
10-*epi*-γ-Eudesmol	1617			1.40
1-*epi*-Cubenol	1627		0.33	
Cis-cadin-4-en-7-ol	1631		1.84	
Caryophylla-4(14),8(15)-dien-5-ol[b]	1636		0.55	
γ-Eudesmol (3)	1638			23.70
Hinesol	1641			0.89
Cubenol	1641		0.42	
β-Eudesmol (2)	1660			51.60
α-Eudesmol (4)	1662			14.56
14-hydroxy-9-*epi*-(*E*)-caryophyllene	1670		2.50	
Ishwarone	1679		2.01	
14-oxy-α-muurolene	1770		0.22	
Carissone	1926			0.18
Monoterpenes identified		70.52	0.08	
Sesquiterpenes identified		29.25	90.56	97.13

[a] Calculated retention index.
[b] Correct isomer not identified.

For the species selected, a literature search produced only a phytochemical study of the essential oil of *G. blepharophylla* (Maia et al., 2005). The major constituent identified in that study is the same determined now, caryophyllene oxide (**1**), which was analysed by ^1H and ^{13}C NMR spectroscopy. In the study reported here, more constituents were identified than in the previous work.

The essential oil of *G. friesiana* contained certain compounds that were not observed in the other oils. However, some constituents, including α-selinene and spathulenol, present in this oil have been found in other species of the family Annonaceae (Maia et al., 2005). The compounds (**2**), (**3**), and (**4**) have been identified in the essential oil of *Hexalobus crispiflorus* (Annonaceae) (Boyom et al., 2003). This is the second isolation of these compounds in the essential oil reported in the Annonaceae family. We also observed that the compounds (**1**), (*E*)-caryophyllene, γ-gurjunene, δ-cadinene, β-selinene, α-*trans*-bergamotene and humulene epoxide II were found in oils of both *G. hispida* and *G. blepharophylla*. These results are in agreement with previous reports for the family Annonaceae (Leboeuf et al., 1982).

Recent studies (Maia et al., 2005; Founier et al., 1997) indicated that (**1**) and spathulenol are also important volatile compounds of the leaf oils of *Guatteria ouregou*, *G. sagotiana* and *G. wachenheimi*, occurring in French Guiana. The genus *Guatteriopsis* is cited in the literature as a segregated genus from *Guatteria*, because it is unique in its peculiar rhomboid petals that are glabrated on the inner surface. Based on the oil composition analysis of this genus, we observed that they are also very similar, as seen in their systematic position within the Annonaceae when compared with literature data (Maia et al., 2005; Founier et al., 1997).

The compounds (**1**), (*E*)-caryophyllene and spathulenol have been reported in some *Xylopia*, *Guatteria*, *Hexalobus*, *Pachypodanthium* and *Duguetia* species (Maia et al., 2005; Founier et al., 1997; Boyom et al., 2003; Fechine et al., 2002; Lima et al., 2003).

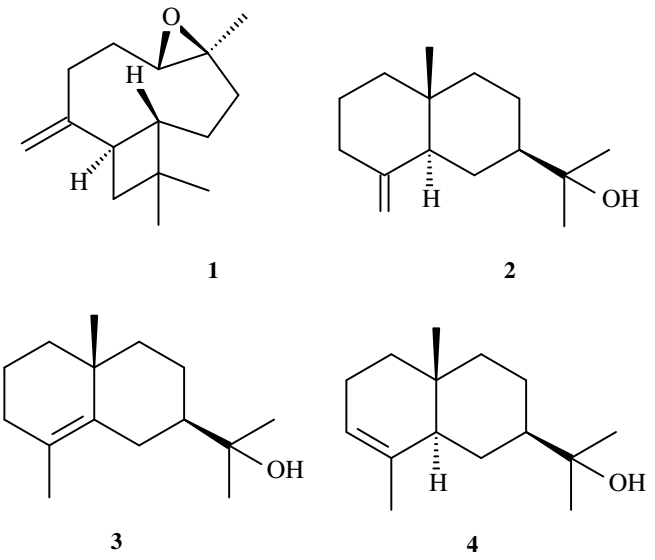

Fig. 1. Structures of compounds isolated from the essential oil of *G. friesiana* and *G. blepharophylla*.

Table 2
Antimicrobial activity of the essential oils, pure compounds, mixtures A and B from *Guatteriopsis* species

Essential oil/ pure compounds	Minimal inhibitory concentration ($\mu g\ mL^{-1}$)										
	B. subtilis	*S. epidermides*	*E. faecium*	*E. hirae*	*E. coli*	*M. luteus*	*P. aeruginosa*	*R. equi*	*S. choleraesuis*	*S. aureus*	*C. albicans*
G. hispida	[f]	[f]	[f]	[f]	[f]	[f]	[f]	50	[f]	[f]	[f]
G. blepharophylla	[f]	[f]	[f]	[f]	[f]	1000	[f]	50	[f]	1000	700
G. friesiana	60	100	250	100	900	125	900	50	600	125	125
Caryophyllene oxide(1)	[e]	[e]	[e]	[e]	[e]	[f]	[e]	500	[f]	[e]	600
β-eudesmol (2)	300	600	[f]	300	[f]	[f]	[f]	125	[f]	[f]	125
γ-eudesmol (3)	800	700	[f]	[f]	[f]	800	300	800	[e]	600	500
α-eudesmol (4)	600	700	[e]	600	[e]	300	200	100	[e]	250	125
mixture A[a]	100	300	[e]	[e]	[e]	400	[e]	400	[e]	250	800
α-pinene	[e]	[e]	[e]	[e]	[e]	[e]	[e]	500	[e]	[e]	100
β-pinene	[e]	[e]	[e]	[e]	[e]	[e]	[e]	800	[e]	[e]	500
(*E*)-caryophyllene	[e]	[e]	[e]	[e]	[e]	[e]	[e]	600	[e]	[e]	250
mixture B[b]	[e]	[e]	[e]	[e]	[e]	[e]	[e]	700	[e]	[e]	700
Chloramphenicol[c]	20	40	70	120	40	50	850	40	60	20	
Nystatin[d]											50

[a] Mixture of β-eudesmol, γ-eudesmol and α-eudesmol (1.0:0.5:0.3 ratio).
[b] Mixture of β-pinene, α-pinene and (*E*)-caryophyllene (1.0:0.8:0.5 ratio).
[c] Drug reference for bacteria.
[d] Drug reference for fungus.
[e] Not tested.
[f] >1000 $\mu g\ mL^{-1}$.

They are considered as chemotaxonomic markers for these Annonaceae genera (Founier et al., 1997; Fechine et al., 2002;Lima et al., 2003).

The results obtained in the evaluation of the antimicrobial activity of the essential oils are shown in Table 2. The most effective oil was the oil of *G. friesiana*, which exhibited a significant antimicrobial activity for all microorganisms tested, displaying strong activity for *Bacillus subtilis*, *Staphylococcus epidermides, Enterococcus hirae, Candida albicans*, *Micrococcus luteus*, *Staphylococcus aureus* and *Rhodococcus equi* with MIC values of 60, 100, 100, 125, 125, 125 and 50 $\mu g\ mL^{-1}$, respectively. *G. blepharophylla* showed activity for *C. albicans*, *M. luteus* and *S. aureus* with MIC of 700, 1000 and 1000 $\mu g\ mL^{-1}$, respectively and presented strong activity for *R. equi* with MIC of 50 $\mu g\ mL^{-1}$. *G. hispida* demonstrated selective activity for *R. equi* with MIC value of 50 $\mu g\ mL^{-1}$.

The potent activity of the oil of *G. friesiana* might be attributed to its high sesquiterpene content (97.13%), and similarly for the oil of *G. blepharophylla* (90.56%). The activity of *G. friesiana* could result from the sesquiterpenoids (**2**) (51.60%), (**3**) (23.70%) and (**4**) (14.56%), which represent 89.86% of the total of the oil analyzed. However, the bioassays performed with these isolated sesquiterpenoids did not show the same activity as observed for the crude oil (Table 2). In view of the possibility of a synergistic effect, a mixture of these compounds, in the proportions in which they were found in the oil (mixture A), was also tested, and showed lower activity than the crude oil (Table 2).

Comparing the activity of mixture A with the isolated compounds, a synergistic effect was observed only for the microorganisms *B. subtilis* and *Staphylococcus epidermides* (Table 2). In contrast, each pure compound was more active against the yeast *C. albicans* than was mixture A (800 $\mu g\ mL^{-1}$). In this case, the activity of the crude oil was the same as observed when (**2**) and (**4**) were tested alone (125 $\mu g\ mL^{-1}$), suggesting that the activity of the crude oil is conferred by these two compounds.

For the microorganisms *M. luteus*, *R. equi* and *C. albicans,* the presence of (**3**) apparently is the reason for the lower activity observed for mixture A, compared with pure (**2**) and (**4**) (Table 2).

According to the results, the isolated compounds from *G. friesiana* are not responsible for the activity against *Enterococcus faecium* or *Escherichia coli* observed for the crude essential oil.

Finally, the results related to *Enterococcus hirae* and *Pseudomonas aeruginosa* show that although the crude oil and at least one compound from *G. friesiana* were active, mixture A was not active over the range of concentrations tested (up to 1000 $\mu g\ mL^{-1}$).

β-eudesmol (**2**) is already known to exhibit several biological activities such as a diuretic, hypotensive activity, antihepatotoxic or antiepileptic agent, and inseticidal and antimicrobial properties (Kusuma et al., 2004; Ding et al., 2000; Arora et al., 1967; Kiso et al., 1983; Chiou et al., 1997; Marinho et al., 2005; Miyakado et al., 1976). Sesquiterpenoids and their derivatives are credited with various biological actions, including antiasthmatic, antibacterial, antifungal, anti-inflammatory, and antineoplastic activities (Founier et al., 1997).

The selective activity of *G. hispida* could be attributed to α- and β-pinene and (*E*)-caryophyllene, and for *G. blepharophylla* can be attributed to (**1**), which are already known to exhibit antimicrobial activity (Boyom et al., 2003; Juven et al., 1994; Harbone and Williams, 1995; Kim et al., 1995; Cimanga et al., 2002; Shunying et al., 2005; Sibanda et al., 2004).

The pure main compounds from *G. hispida* (α-pinene, β-pinene and (*E*)-caryophyllene) showed activity against the microorganisms *R. equi* and *C. albicans*. However, the MICs observed for *R. equi* for the isolated compounds and mixture B (mixture of main compounds, in the proportions in which they were found in the oil) were higher than that determined by the crude oil (Table 2), probably as a result of a synergistic action of other compounds present in the oil. This likely synergistic action was also observed for the *G. blepharophylla* essential oil and (**1**).

3. Conclusion

This is the first report on the analysis of volatile constituents, antimicrobial activities, isolation and characterization of major compounds from leaves of *G. friesiana*, *G. hispida* and *G. blepharophylla*.

Interesting results were obtained from the assays, suggesting that different responses can be obtained from the use of the crude oil, isolated compounds or a mixture of them, depending on the microorganism considered, and of synergistic effect.

Although the oils in our study showed potent activity for *R. equi*, a bacterium responsible for pneumonia in animals and humans, neither the major isolated sesquiterpenoids nor their mixture showed the same activity.

The isolated and identified compounds of essential oils are very important for chemotaxonomic of the family Annonaceae,

especially of the *Guatteria* group, which posses four genera (*Guatteria*, *Guatteriopsis*, *Guatteriella* and *Heteropetalum*) that present some little differences among them in the morphological characters.

4. Experimental

4.1. General

NMR spectra were recorded on a Bruker ARX-200 Spectrometer (200 MHz for ^{1}H and 50 MHz for ^{13}C) using deuterated chloroform as solvent. The chemical shift values are reported in parts per million with reference to TMS. GC/FID analysis was carried out on Hewlett Packard 6890 GC system with FID and GC/MS analysis on Hewlett Packard 6890 GC coupled with a model 5973 selective mass detector.

4.2. Plant material

The leaves of *G. blepharophylla* and *G. friesiana* were collected at the Federal University of Amazonas (UFAM), Manaus, AM, Brazil in January 2005. The leaves of *G. hispida* were collected at the Adolpho Ducke Reserve, in the vicinity of Manaus, AM, Brazil in February 2005. Random collections of leaves were taken regardless of the age or plant, and were combined into 1 sample. The plant samples were identified by Annonaceae specialist Dr. Antônio Carlos Webber from the Federal University of Amazonas (UFAM). Voucher specimens of *G. blepharophylla* (no. 7340), *G. friesiana* (no. 7341) and *G. hispida* (no. 7707) were deposited in the Herbarium of the Department of Biology, UFAM, Manaus, Amazonas, Brazil.

4.3. Isolation of essential oils

Three samples of leaves (250 g each) of each species of *Guatteriopsis* were dried at room temperature for 5 days and then submitted to hydrodistillation for 4 h, in a Clevenger-type apparatus. The oils were dried over anhydrous Na_2SO_4 and their percentage contents were calculated on the basis of the dry weight of plant material. The oils were stored at 4 °C until further analysis. All specimens were obtained from flowering plants.

4.4. GC/FID analysis

The analysis of the volatile compounds was performed on a Hewlett Packard 6890 GC system with a fused capillary column (30 m × 0.25 mm × 0.25 μm, SA-5, Crossbond 5% phenyl-95% dimethylpolysiloxane, Sigma–Aldrich) directly coupled to a flame ionization detector. Conditions of injection were modified from Adams (2001): injector temperature 240 °C; oven temperature program of 60–300 °C at a rate of 3 °C/min; split 20:1 during 1.50 min, carrier gas He: 1 mL/min, constant flow; sample volume 1 μL.

4.5. GC/MS analysis

The GC/MS analyses were performed in EI mode on a Hewlett Packard-6890 GC system with a fused capillary column (30 m × 0.25 mm × 0.25 μm, HP-5MS, Crossbond 5% phenyl-95% dimethylpolysiloxane) directly coupled to on Hewlett Packard 5973 selective mass detector. The conditions of injection were the same as described above. The mass spectrometer was operated at 70 eV. 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, 2001). The retention indices (RI) were calculated according to van den Dool and Kratz (1963).

4.6. Essential oil fractionation

4.6.1. Guatteriopsis blepharophyla

The essential oil (200 mg) was fractionated by silica gel 60 column chromatography (1.5 × 43.0 cm) (Merck, 0.063–0.200 mm) using petroleum ether with increasing amounts of CH_2Cl_2 (0%, 5%, 10%, 20%, 50% and 80%) followed by CH_2Cl_2 with increasing amounts of EtOAc (0%, 5%, 10%, 20% and 50%) as eluent. Forty-five fractions (25 mL) were obtained. The eluted fractions were evaluated and pooled by TLC analysis. Fraction 20–25 (50 mg) afforded caryophyllene oxide (**1**) pure enough for NMR spectroscopic identification.

4.6.2. G. friesiana

The essential oil (300 mg) was subjected to the same conditions as above. Forty fractions (30 mL) were obtained. The eluted fractions were evaluated and pooled by TLC analysis. Additional chromatographic separation of fractions 24–28 (50 mg) was carried out by preparative TLC and 1% $AgNO_3$ doped silica gel, eluted with petroleum ether–EtOAc (80:20, v/v), to provide β-eudesmol (**2**), γ-eudesmol (**3**) and α-eudesmol (**4**), pure enough for NMR spectroscopic identification.

4.6.3. Caryophyllene oxide (*1*)

Colourless oil (50.0 mg). ^{1}H and ^{13}C NMR spectroscopic data: same as the data reported in Rharivelomanana et al. (1995). EI-MS data: same as the data reported in Sköld et al. (2006) and Rharivelomanana et al. (1995).

4.6.4. β-Eudesmol (*2*)

Amorphous solid (15.0 mg). ^{1}H, ^{13}C NMR spectroscopic and EI-MS data: same as the data reported in Kusuma et al. (2004) and Rharivelomanana et al. (1995).

4.6.5. γ-Eudesmol (*3*)

Colourless oil (8.0 mg). ^{1}H and ^{13}C NMR spectroscopic data: same as the data reported in Rharivelomanana et al. (1995). EI-MS data: same as the data reported in van Beek et al. (1989).

4.6.6. α-Eudesmol (*4*)

Amorphous solid (6.4 mg). ^{1}H and ^{13}C NMR spectroscopic data: same as the data reported in Rharivelomanana et al. (1995). EI-MS data: same as the data reported in van Beek et al. (1989).

4.7. Microorganisms cultures

Growth inhibitory activity of the essential oils was tested against 11 microorganisms (*B. subtilis* ATCC 5061, *C. albicans* ATCC 10231, *E. faecium* CCT 5079, *E. hirae* ATCC 10541, *E. coli* ATCC 11775, *M. luteus* ATCC 4698, *P. aeruginosa* ATCC 13388, *R. equi* ATCC 6939, *Salmonella choleraesuis* ATCC 10708, *S. aureus* ATCC 6538 and *S. epidermides* ATCC 12228). The microorganisms were obtained from the Tropical Culture Collection (TCC) of the André Tosello Foundation, Campinas, São Paulo, Brazil. The microorganisms were selected for their medical importance; they are systematically used in our laboratory as part of a screening program aiming at the identification of natural products with potential to be exploited as new antimicrobial drugs. The growth inhibitory activity of the caryophyllene oxide (**1**), β-eudesmol (**2**), γ-eudesmol (**3**), α-eudesmol (**4**) (all isolated from the oils), α-pinene, β-pinene, (*E*)-caryophyllene (from Aldrich Chemical Company, Milwaukee, Wisconsin, USA), mixture A (β-eudesmol, γ-eudesmol, α-eudesmol,

1.0:0.5:0.3 ratio) and mixture B (β-pinene, α-pinene, (*E*)-caryophyllene, 1.0:0.8:0.5 ratio) were also evaluated.

4.7.1. Determination of minimal inhibitory concentration (MIC)

The bacterial strains were grown overnight at 36 °C in Nutrient Agar (Merck), while *C. albicans* was grown in Saboraud Dextrose Agar. Inoculum for the assays was prepared by diluting scraped cell mass in 0.85% NaCl solution, adjusted to McFarland scale 0.5 and confirmed by spectrophotometrical reading at 580 nm. Cell suspensions were finally diluted to 10^4 UFC mL^{-1} for use in the activity assays. Minimal Inhibitory Concentration (MIC) tests were carried out according to Ellof (1998), using Müller-Hinton broth on a tissue culture test plate (96 wells). Each vegetal material was tested in duplicate. The stock solutions of essential oils, pure compounds and mixtures A and B were diluted and transferred into the first well, and serial dilutions were performed so that concentrations in the range of 1.0–0.016 mg mL^{-1} (1.0, 0.5, 0.25, 0.125, 0.063, 0.032 and 0.016 mg mL^{-1}) were obtained. Chloramphenicol and nystatin (Merck) were used as the reference antibiotic control in the range of 0.25–0.002 mg mL^{-1} (0.25, 0.125, 0.063, 0.032, 0.016, 0.008, 0.004 and 0.002 mg mL^{-1}). The inoculum was added to all wells and the plates were incubated at 36 °C for 48 h. Antimicrobial activity was detected by adding 20 μL of 0.5% TTC (triphenyl tetrazolium chloride, Merck) aqueous solution. MIC was defined as the lowest concentration of the essential oils, pure compounds and mixtures A and B that inhibited visible growth, as indicated by TTC staining (dead cells are not stained by TTC).

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References

Adams, R.P., 2001. Identification of Essential oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois.

Arora, C.K., Arora, R.B., Mesta, C.K., Shanbag, S.N., Seshari, R., Maheshari, M.L., Battacharya, S.C., 1967. Hypotensive activity of β-eudesmol and some related sesquiterpenes. Ind. J. Med. Res. 55, 463–472.

van Beek, T.A., Kleis, R., Posthumus, M.A., van Veldhuizen, A., 1989. Essential oil of *Amyris balsamifera*. Phytochemistry 28, 1909–1911.

Boyom, F.F., Ngouana, V., Zollo, P.H.A., Menut, C., Bessiere, J.M., Gut, J., Rosenthal, P.J., 2003. Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. Phytochemistry 64, 1269–1275.

Braga, R., 1976. Plantas do Nordeste especialmente do Ceará, 4ª ed. Editora Universitária UFRN, Natal-Rio Grande do Norte.

Castedo, L., Granja, J.A., Lera, A.R., Villaverde, M.C., 1991. Alkaloids from *Guatteria goudotiana*. Phytochemistry 30, 2781–2783.

Chiou, L.C., Ling, J.Y., Chang, C.C., 1997. Chinese herbs constituent β-eudesmol alleviated the electroshock seizures in mice and electrographic seizures in rat hippocampal slices. Neurosci. Lett. 231, 171–174.

Cimanga, K., Kambu, K., Tona, L., Apers, S., Bruyne, I., Hermans, N., Totté, J., Pieters, L., Vlietinck, A.J., 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J. Ethnopharmacol. 79, 213–220.

Ding, H.Y., Wu, Y.C., Lin, H.C., 2000. Phytochemical and pharmacological studies on Chinese changzhu. J. Chinese Chem. Soc. 47, 561–566.

Ellof, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64, 711–713.

Fechine, I.M., Navarro, V.R., Da Cunha, E.V.L., Silva, M.S., Maia, J.G.S., Barbosa-Filho, M., 2002. Alkaloids and volatile constituents from *Duguetia flagellaris*. Biochem. Syst. Ecol. 30, 267–269.

Founier, G., Hadjiakhoondi, A., Leboeuf, M., Cavé, A., 1997. Essential oils of Annonaceae. Part. VIII. Volatile constituents of the essential oils from three *Guatteria* species. J. Essent. Oil Res. 9, 275–278.

Gemtchújnicov, I.D., 1976. Manual de Taxonomia Vegetal: plantas de interesse econômico. Ed. Agronômica Ceres, São Paulo-SP.

Harbone, J.B., Williams, C.A., 1995. Anthocyanins and other flavonoids. Nat. Prod. Rep. 7, 453–455.

Hutchinson, J., 1964. The Genera of Flowering Plants, vol. 1. University Press, Oxford.

Juven, B.J., Kanner, J., Schved, F., Weisslowic, H., 1994. Factors that interact with antibacterial action of thyme essential oil and its active constituents. J. Appl. Bacteriol. 76, 626–631.

Kim, J., Marshall, M.R., Wei, C., 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. J. Agric. Food Chem. 43, 2839–2845.

Kiso, Y., Tohkin, M., Hikino, H., 1983. Antihepatotoxic principles of Atractylodes rhizomes. J. Nat. Prod. 46, 651–654.

Kusuma, I.W., Ogawa, T., Itoh, K., Tachibana, S., 2004. Isolation and identification of an antifungal sesquiterpene alcohol from *Amboyna* wood. Pak. J. Biol. Sci. 7, 1735–1740.

Leboeuf, M., Cavé, A., Bhaumik, P.K., Mukherjee, B., Mukherjee, R., 1982. phytochemistry of the Annonaceae. Phytochemistry 12, 2783–2913.

Lima, M.A., Fechine, I.M., Silva, M.S., Maia, J.G.S., Cunha, E.V.L., Barbosa-Filho, J.M., 2003. Alkaloids and volatile constituents from *Guatteria juruensis*. Biochem. Syst. Ecol. 31, 423–425.

Maia, J.G.S., Andrade, E.H.A., Carreira, L.M.M., Oliveira, J., Araújo, J.S., 2005. Essential oils of the Amazon *Guatteria* and *Guatteriopsis* species. Flavour Frag. J. 20, 478–480.

Marinho, C.G.S., Della Lucia, T.M.C., Guedes, R.N.C., Ribeiro, M.M.R., Lima, E.R., 2005. β-eudesmol-induced aggression in the leaf-cutting ant *Atta sexdens rubropilosa*. Entomol. Exper. Applic. 117, 89–93.

Miyakado, M., Kato, T., Ohno, N., Mabry, T.J., 1976. Pinocembrin and (+)-β-eudesmol from *Hymenoclea monogyra* and *Bacharis glutinosa*. Phytochemistry 15, 846.

Raharivelomanana, P., Bianchini, J.P., Cambon, A., Azzaro, M., Faure, R., 1995. Two-dimensional NMR of sesquiterpenes. Magn. Reson. Chem. 33, 233–235.

Ribeiro, J.E.L.S., Maas, P.J.M., Maas, H., Miralha, J.M., Webber, A., 1999. Annonaceae. In: Ribeiro, J.E.L.S., Hopkins, M.J.G., Vicentini, A., Sothers, C.A., Costa, M.A.S., Brito, J.M., Souza, M.A.D., Martins, L.H.P., Lohmann, L.G., Assunção, P.A.C.L., Pereira, E.C., Silva, C.F., Mesquita, M.R., Procópio, L.C. (Eds.), Flora da Reserva Ducke Guia de Identificação das plantas vasculares de uma floresta de terra-firme na Amazônia Central. DFID (Departamento for International Development), INPA, Manaus – Amazonas, pp. 121–135.

Rupprecht, J.K., Hui, Y.H., MacLaughlin, J.L., 1990. Annonaceous acetogenins: a review. J. Nat. Prod. 53, 237–278.

Shunying, Z., Yang, Y., Huaidong, Y., Yue, Y., Guolin, Z., 2005. Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. J. Ethnopharmacol. 96, 151–158.

Sibanda, S., Chigwada, G., Poole, M., Gwebu, E.T., Noletto, J.A., Schmidt, J.M., Rea, A.I., Setzer, W.N., 2004. Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. J. Ethnopharmacol. 92, 107–111.

Sköld, M., Kalberg, A.T., Matura, M., Börje, A., 2006. The fragrance chemical β-caryophyllene-air oxidation and skin sensitization. Food Chem. Toxicol. 44, 538–545.

van den Dool, H., Kratz, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. J. Chromatogr. A 11, 463–471.