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## Essential oils from branches of *Bocageopsis*, *Guatteria* and *Unonopsis* species: chemical composition and antibacterial activity

### Óleos essenciais dos galhos de espécies de *Bocageopsis*, *Guatteria* e *Unonopsis*: composição química e atividade antibacteriana

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#### Joelma Moreira Alcântara

ORCID: <https://orcid.org/0000-0003-0240-4837>

Universidade Federal do Amazonas, Brasil

E-mail: [jomalc@yahoo.com.br](mailto:jomalc@yahoo.com.br)

#### Juliana Mesquita V. M. de Lucena

ORCID: <https://orcid.org/0000-0002-3771-6905>

Instituto Federal do Amazonas, Brasil

E-mail: [jlucena@ifam.edu.br](mailto:jlucena@ifam.edu.br)

#### Pedro Igor Lima Soares

ORCID: <https://orcid.org/0000-0002-5218-1750>

Hospital Universitário Getúlio Vargas, Brasil

E-mail: [pedro\\_igor51@hotmail.com](mailto:pedro_igor51@hotmail.com)

#### Marcia Ortiz M. Marques

ORCID: <http://orcid.org/0000-0001-8270-4308>

Instituto Agronômico de Campinas, Brasil

E-mail: [mortiz@iac.sp.gov.br](mailto:mortiz@iac.sp.gov.br)

#### Maria da Paz Lima

ORCID: <http://orcid.org/0000-0002-0255-0693>

Instituto Nacional de Pesquisas da Amazônia, Brasil

E-mail: [mdapaz@inpa.gov.br](mailto:mdapaz@inpa.gov.br)

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### ABSTRACT

Among the Amazonian species of the family Annonaceae, *Bocageopsis multiflora* (Mart.) R.E. Fr., *Guatteria blepharophylla* Mart., *Guatteria guianensis* (Aubl.) R.E. Fr. and *Unonopsis guatterioides* (A. DC.) R.E. Fr. present promising essential oils and biological potential. These species, which occur in *terra firme* forest, have had the essential oils (EOs) from their leaves previously evaluated, but there is a lack of studies regarding their branches (except for the species *B. multiflora*). Thus, in the present study, the essential oils of the branches of four species were investigated using GC-FID and GC-MS, and their antibacterial activity was also evaluated. In *B. multiflora*, the main constituents were exo-2-norborneol acetate ( $10.3 \pm 0.4$ ) and *ar*-curcumene ( $10.2 \pm 0.3\%$ ). *Ar*-curcumene ( $10.0 \pm 0.3\%$ ), caryophyllene oxide ( $8.4 \pm 0.1\%$ ) and (*Z,Z*)-farnesol ( $7.2 \pm 0.2\%$ ) were predominant in the EO of *G. guianensis*. Caryophyllene oxide ( $51.0 \pm 0.1\%$ ) was the predominant constituent of the essential oil from *G. blepharophylla*. *U. guatterioides* showed the phenylpropanoid elemicin ( $71.7 \pm 0.3\%$ ) as its main constituent. The EO of *G. guianensis* showed strong bactericidal activity against *Enterococcus faecalis*.

**Keywords:** Annonaceae; Terpenes; Phenylpropanoid; *Enterococcus faecalis*

## RESUMO

Entre as espécies amazônicas da família Annonaceae, *Bocageopsis multiflora* (Mart.) R.E. Fr., *Guatteria blepharophylla* Mart., *Guatteria guianensis* (Aubl.) R.E. Fr. e *Unonopsis guatterioides* (A. DC.) R.E. Fr. apresentam em óleos essenciais (OEs) promissores e potencial biológico. Essas espécies, ocorrentes em floresta de *terra firme*, já tiveram seus óleos essenciais de folhas previamente avaliados, porém há carência de estudos em galhos (exceto para a espécie *B. multiflora*). Assim, no presente estudo, os óleos essenciais dos galhos de quatro espécies foram investigados por GC-FID e GC-MS, e sua atividade antibacteriana também foi avaliada. Em *B. multiflora*, os constituintes majoritários foram o acetato de exo-2-norborneol ( $10,3 \pm 0,4$ ) e o *ar*-curcumeno ( $10,2 \pm 0,3\%$ ). O *ar*-curcumeno ( $10,0 \pm 0,3\%$ ), óxido de cariofileno ( $8,4 \pm 0,1\%$ ) e (*Z,Z*)-farnesol ( $7,2 \pm 0,2\%$ ) foram os predominantes no OE de *G. guianensis*. O óxido de cariofileno ( $51,0 \pm 0,1\%$ ) foi o principal constituinte do óleo essencial de *G. blepharophylla*. Como o principal constituinte, *U. guatterioides* apresentou o fenilpropanóide elemicina ( $71,7 \pm 0,3\%$ ). O OE de *G. guianensis* apresentou forte atividade bactericida contra *Enterococcus faecalis*.

**Palavras-chaves:** Annonaceae; Terpenos; Fenilpropanoide; *Enterococcus faecalis*

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## INTRODUÇÃO

Several chemical studies have been carried out regarding the essential oils of species of Annonaceae obtained via steam distillation and reviews have been published citing a number of biological activities (Fournier *et al.*, 1999; Cascaes *et al.*, 2022). Among the Amazonian species of Annonaceae, *Bocageopsis multiflora* (Mart.) R.E. Fr., *Guatteria blepharophylla* Mart., *Guatteria guianensis* (Aubl.) R.E. Fr. and *Unonopsis guatterioides* (A. DC.) R.E. Fr. present promising oils and biological potential. These species, which occur in *terra firme* forest, have had their essential oils from leaves previously evaluated, but there is a lack of studies regarding their branches (except for the species *B. multiflora*).

The main constituents of the essential oil (EO) from the leaves of *B. multiflora* related by Soares *et al.*, (2022) were spathulenol (35.14%) and  $\alpha$ -*trans*-bergamotene (31.23%), and, in the branches, the main constituents were  $\alpha$ -*trans*-bergamotene (25.32%),  $\beta$ -selinene (19.57%), and  $\alpha$ -gurjunene (15.42%). Alcântara *et al.* (2017) found spathulenol (20.3%) and  $\beta$ -bisabolene (11.9%) as the predominant constituents of the essential oil of leaves of this species. Oliveira *et al.* (2014) observed that the main constituent of the EO of leaves collected in the rainy season was bisabolene (13.2%), while the main constituent in the dry season was spathulenol (16.2%). These authors noted activity against *Leishmania amazonensis* promastigote form in EO collected in the rainy season (Oliveira *et al.*, 2014).

In studies of the chemical composition of EOs from leaves of Amazonian specimens of *G. blepharophylla*, high levels of caryophyllene oxide were evidenced, i.e., 51% (Alcantara *et al.*, 2017), 69.3% (Costa *et al.*, 2008) and 70% (Aciole *et al.*, 2011). Essential oils with percentages of 51 and 70% exhibited bactericidal activity against *Streptococcus sanguinis* and insecticidal

activity against *Aedes aegypti*, respectively. The species *U. guatterioides* presented  $\alpha$ -copaene (11.26%) as a major constituent (Silva *et al.*, 2015) essential oil of its leaves. However, there is no record of studies regarding the essential oils of *Guatteria guianensis*.

In the present study, the essential oils from the branches of four Amazonian species of the family Annonaceae were investigated using GC/MS analysis, and their antibacterial activity was also evaluated.

## MATERIAL AND METHODS

### Plant material for the extraction of essential oils

Branches of *Bocageopsis multiflora* (voucher 245131) were collected from individuals at the National Institute for Amazonian Research (INPA, Campus Manaus, AM); *Guatteria blepharophylla* (9231), *G. guianensis* (9239) and *Unonopsis guatterioides* (9238) were collected from individuals at the Universidade Federal do Amazonas (UFAM, Campus Manaus, AM). The branches were dried at room temperature under reduced light, then pulverized in a grinder and submitted to hydrodistillation (in triplicate) for four hours using a modified Clevenger-type apparatus. The resulting distilled oils were dried over anhydrous sodium sulphate (Merck) and stored in amber glass bottles in a refrigerator (4 °C).

### Analysis of the essential oils

The essential oils were analyzed using GC-FID (Shimadzu, GC 2010 with flame ionization detector), in a CP-sil 5 CB (Varian) 100% dimethylpolysiloxane fused silica capillary column (15 m x 0.25 mm x 0.25  $\mu$ m). The carrier gas was helium (flow 10 mL.min<sup>-1</sup>), and the operating conditions were temperature at 60-240 °C (3 °C.min<sup>-1</sup>); injection of 1.0  $\mu$ L; sample injection temperature at 250 °C; detector temperature 290 °C; split 1:20. The analysis of volatile constituents was performed by using a gas chromatograph (Shimadzu QP-5000) coupled to a mass spectrometry detector (GC-MS), in a OV-5, fused silica capillary column (30 m x 0.25 mm x 0.25  $\mu$ m) (Ohio Valley Specialty Chemical, Inc.). The analysis conditions of the oven were the same as those used for GC-FID, using the electron impact technique at 70 eV. Each essential oil was analyzed in triplicate via GC-FID and GC-MS. The retention indices (KI) were calculated in relation to the elution times of essential oil compounds and a series of *n*-alkanes (C9-C22), co-injected with the sample in GC-FID. The constituents were identified with the data set of retention indices and mass spectra were compared with the data found in the literature (Adams, 2001) and the NIST 12, NIST62 and WILEY 139 databanks.

### Antibacterial assay

The bacterial strains used for screening were Gram-positive *Staphylococcus aureus* (ATCC6538), *Enterococcus faecalis* (ATCC29212) and *Streptococcus sanguinis* (ATCC10556)

and Gram-negative *Pseudomonas aeruginosa* (ATCC9027), *Salmonella enterica* (ATCC13076) and *Escherichia coli* (ATCC 8739). All microorganisms were made available by the Oswaldo Cruz Foundation (FIOCRUZ, Rio de Janeiro, Brazil). The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) on 96-well culture plates using the microdilution method, starting from a solution at a concentration of 20 mg.mL<sup>-1</sup> in 10% DMSO. A metabolic test was performed by adding 10 µL of resazurin (1%). A solution of chlorhexidine digluconate (2%) served as the positive control, and the negative control was the same solution of DMSO 10% used to solubilize the essential oils. The samples were tested in triplicate and resazurin was used as an indicator of bacterial growth.

## RESULTS AND DISCUSSION

Essential oils from four individuals were obtained with yield of 0.11% ± 0.00 for *B. multiflora* and *G. guianensis*, 0.12% ± 0.02 for *G. blepharophylla* and 0.16 ± 0.00 for *U. guatterioides* (in relation to the weight of the dry matter). The total percentage of identified compounds (95.4-99.8%) in the samples of the four essential oils was high, as shown in Table 1. The essential oils of the two species of *Guatteria* had higher numbers of identified chemical constituents (Table 2).

The sesquiterpenes, mainly oxygenated ones, were predominant in the essential oils of the evaluated species. In the essential oil of *B. multiflora*, the main constituents were the oxygenated monoterpene *exo*-2-norborneol acetate (10.3 ± 0.4) and the sesquiterpene hydrocarbon *ar*-curcumene (10.2 ± 0.3%), in addition to the oxygenated sesquiterpene costol (7.9 ± 0.0%). *Ar*-curcumene (10.0 ± 0.3%), caryophyllene oxide (8.4 ± 0.1%), (*Z,Z*)-farnesol (7.2 ± 0.2%),  $\delta$ -cadinene (6.0 ± 0.2%) and 2,3-dihydrofarnesol (6.0 ± 0.0%) were predominant in the EO of *G. guianensis*. Caryophyllene oxide (51.0 ± 0.1%) was the predominant constituent of the essential oil of *G. blepharophylla*. The EO of *U. guatterioides* showed the lowest number of volatile constituents and the main constituent identified was the phenylpropanoid elemicin (71.7 ± 0.3%). As yet, there are no studies on the essential oils from the branches of *Guatteria guianensis*, *G. blepharophylla*, and *Unonopsis guatterioides*, so ours would be the first.

The high content of elemicin detected in *U. guatterioides* had not yet been registered in Brazilian species of Annonaceae; however, the literature reports high levels of phenylpropanoids such as elemicin and methyl eugenol in Australian species of the family (Brophy *et al.*, 2008).

In this research, the essential oils showed weak inhibition for Gram-negative bacteria (*P. aeruginosa*, *S. enterica* and *E. coli*). The EO of *G. guianensis*, which has a predominance of *ar*-curcumene, caryophyllene oxide, (*Z,Z*)-farnesol,  $\delta$ -cadinene and 2,3-dihydrofarnesol, showed strong activity against *Enterococcus faecalis*. The EO of *Bocageopsis multiflora*, which has a predominance of *exo*-2-norborneol acetate, *ar*-

curcumene and costol, showed significant bactericidal activity against *Enterococcus faecalis* and *Streptococcus sanguinis*. In previous studies with *B. multiflora* leaves, essential oils were active against *Streptococcus sanguinis* (Alcântara *et al.*, 2017), but the predominant constituents identified were different from those detected in the branches of this species.

**Table 1.** Percentage composition of the essential oils of branches from *B. multiflora* (BM), *G. blepharophylla* (GB), *G. guianensis* (GG) and *U. guatterioides* (UG)

Compounds	RI	BM	GB	GG	UG
$\alpha$ -pinene	930			0.5 $\pm$ 0.0	
$\beta$ -pinene	973			0.6 $\pm$ 0.2	
<i>cis</i> -thujone	1101	1.1 $\pm$ 0.0			
linalool	1096			0.2 $\pm$ 0.4	0.4 $\pm$ 0.2
<i>exo</i> -2-norborneol acetate	1126	<b>10.3</b> $\pm$ 0.4	1.4 $\pm$ 0.0		1.0 $\pm$ 0.0
terpinen-4-ol	1179	0.5 $\pm$ 0.3			
$\alpha$ -terpineol	1187			0.5 $\pm$ 0.0	
<i>trans</i> -carveol	1211	0.3 $\pm$ 0.3			
$\beta$ -elemene	1389	2.2 $\pm$ 0.2	1.3 $\pm$ 0.0	0.2 $\pm$ 0.2	
( <i>Z</i> )-caryophyllene	1403			0.2 $\pm$ 0.1	
$\alpha$ - <i>cis</i> -bergamotene	1410	0.9 $\pm$ 0.0			
$\beta$ -caryophyllene	1416	1.6 $\pm$ 0.1	5.0 $\pm$ 0.1	0.7 $\pm$ 0.2	
$\alpha$ - <i>trans</i> -bergamotene	1433	0.2 $\pm$ 0.0		0.9 $\pm$ 0.2	
aromadendrene	1438	2.7 $\pm$ 0.1			
$\alpha$ -guaiene	1440	0.2 $\pm$ 0.0			
$\alpha$ -humulene	1453		0.6 $\pm$ 0.1	0.3 $\pm$ 0.0	
$\gamma$ -gurjunene	1471	0.5 $\pm$ 0.3			
<i>trans</i> -cadinan-1(6),4-diene	1473	1.3 $\pm$ 0.3	0.5 $\pm$ 0.0		
$\gamma$ -muurolene	1478				0.4 $\pm$ 0.0
<i>ar</i> -curcumene	1482	<b>10.2</b> $\pm$ 0.3	0.5 $\pm$ 0.0	<b>10.0</b> $\pm$ 0.3	
germacrene D	1483		0.5 $\pm$ 0.0		0.7 $\pm$ 0.0
$\alpha$ -selinene	1492	2.9 $\pm$ 0.2	2.1 $\pm$ 0.2	4.7 $\pm$ 0.4	0.9 $\pm$ 0.1
$\alpha$ -muurolene	1497	0.2 $\pm$ 0.3			
$\beta$ -bisabolene	1505	5.3 $\pm$ 0.0	0.4 $\pm$ 0.2	3.7 $\pm$ 0.2	0.4 $\pm$ 0.1
$\gamma$ -cadinene	1511		0.7 $\pm$ 0.0	0.3 $\pm$ 0.2	1.1 $\pm$ 0.1
$\delta$ -cadinene	1524	0.9 $\pm$ 0.2	1.4 $\pm$ 0.1	6.0 $\pm$ 0.2	0.6 $\pm$ 0.2
10- <i>epi</i> -cubebol	1534			0.4 $\pm$ 0.2	
$\alpha$ -calacorene	1540	1.9 $\pm$ 0.2	1.2 $\pm$ 0.1	0.5 $\pm$ 0.3	0.5 $\pm$ 0.0
elemol	1545	1.6 $\pm$ 0.2		0.3 $\pm$ 0.2	
<i>cis</i> -murol-5-en-4- $\beta$ -ol	1550	0.6 $\pm$ 0.1	4.6 $\pm$ 0.0	0.6 $\pm$ 0.2	
elemicine	1553	0.3 $\pm$ 0.3		1.1 $\pm$ 0.0	<b>71.7</b> $\pm$ 0.3
germacrene B	1556	0.6 $\pm$ 0.2		2.3 $\pm$ 0.1	
$\beta$ -calacorene	1565	0.6 $\pm$ 0.1	2.0 $\pm$ 0.1	0.6 $\pm$ 0.0	
spathulenol	1574	5.2 $\pm$ 0.0	4.5 $\pm$ 0.1	0.6 $\pm$ 0.0	4.6 $\pm$ 0.0
caryophyllene oxide	1580	3.4 $\pm$ 0.1	<b>51.0</b> $\pm$ 0.1	8.4 $\pm$ 0.1	1.8 $\pm$ 0.1
globulol	1585	0.2 $\pm$ 0.1			
$\beta$ -copaen-4- $\alpha$ -ol	1587	1.8 $\pm$ 0.3			
<i>allo</i> -cedrol	1590	0.4 $\pm$ 0.1			
viridiflorol	1593	0.2 $\pm$ 0.3		1.0 $\pm$ 0.2	0.7 $\pm$ 0.2
guaiol	1598	2.2 $\pm$ 0.1	0.8 $\pm$ 0.3	0.3 $\pm$ 0.1	
$\beta$ -oplophenone	1604		3.6 $\pm$ 0.3	0.9 $\pm$ 0.0	
humulene epoxide II	1605	2.4 $\pm$ 0.1	0.7 $\pm$ 0.0		0.5 $\pm$ 0.3
<i>cis</i> -isolongifolanono	1611	0.7 $\pm$ 0.2		3.4 $\pm$ 0.1	1.3 $\pm$ 0.2

$\alpha$ -corocalene	1618	0.5 $\pm$ 0.2		0.2 $\pm$ 0.2	
10- <i>epi</i> - $\gamma$ -eudesmol	1622	0.5 $\pm$ 0.3		0.2 $\pm$ 0.0	
1- <i>epi</i> -cubenol	1625	0.1 $\pm$ 0.3	2.8 $\pm$ 0.3	0.2 $\pm$ 0.1	2.7 $\pm$ 0.3
$\gamma$ -eudesmol	1628	0.2 $\pm$ 0.0	0.6 $\pm$ 0.3	2.6 $\pm$ 0.0	
<i>cis</i> -cadin-4-en-7-ol	1632	2.0 $\pm$ 0.0	1.3 $\pm$ 0.2	1.3 $\pm$ 0.1	
<i>epi</i> - $\alpha$ -cadinol	1636	0.7 $\pm$ 0.0	0.8 $\pm$ 0.0	0.6 $\pm$ 0.0	0.7 $\pm$ 0.1
<i>epi</i> - $\alpha$ -muurolol	1642	0.4 $\pm$ 0.0		0.7 $\pm$ 0.0	1.3 $\pm$ 0.0
$\beta$ -eudesmol	1645	0.3 $\pm$ 0.1	0.5 $\pm$ 0.2	0.7 $\pm$ 0.4	0.9 $\pm$ 0.0
$\alpha$ -eudesmol	1650	2.1 $\pm$ 0.3	0.4 $\pm$ 0.3	0.9 $\pm$ 0.0	6.5 $\pm$ 0.1
valerianol	1653			1.9 $\pm$ 0.2	
$\alpha$ -cadinol	1654	1.2 $\pm$ 0.0	0.9 $\pm$ 0.1	1.4 $\pm$ 0.0	
7- <i>epi</i> - $\alpha$ -eudesmol	1657	1.9 $\pm$ 0.2	2.5 $\pm$ 0.1	0.3 $\pm$ 0.1	
<i>cis</i> -calamene-10-ol	1662	1.2 $\pm$ 0.1		0.3 $\pm$ 0.0	
14-hydroxy-9- <i>epi</i> -( <i>E</i> )-caryophyllene	1667	1.2 $\pm$ 0.3	4.6 $\pm$ 0.2	1.2 $\pm$ 0.2	
cadalene	1670	1.4 $\pm$ 0.0	0.6 $\pm$ 0.3	1.1 $\pm$ 0.0	
guaia-3,10(14)-dien-11-ol	1672	0.8 $\pm$ 0.2	0.7 $\pm$ 0.1	1.7 $\pm$ 0.0	
khusinol	1675	0.5 $\pm$ 0.2			
helifolenol C	1679	0.3 $\pm$ 0.0		0.9 $\pm$ 0.0	
<i>epi</i> - $\alpha$ -bisabolol	1681	1.4 $\pm$ 0.3	0.5 $\pm$ 0.3	3.6 $\pm$ 0.2	
$\alpha$ -bisabolol	1682	1.2 $\pm$ 0.0		0.5 $\pm$ 0.3	
2,3-dihydrofarnesol	1687	1.4 $\pm$ 0.2		6.0 $\pm$ 0.0	
acorenone	1690	0.3 $\pm$ 0.0			
( <i>Z,Z</i> )-farnesol	1692	0.6 $\pm$ 0.1		7.2 $\pm$ 0.2	
amorpha-4,9-dien-2-ol	1698			0.3 $\pm$ 0.1	
caryophyllene acetate	1701	0.7 $\pm$ 0.2		0.4 $\pm$ 0.2	
amorpha-4.9-dien-14-al	1702	0.4 $\pm$ 0.2		0.4 $\pm$ 0.1	
14-hydroxy- $\alpha$ -humulene	1708	1.1 $\pm$ 0.1		2.7 $\pm$ 0.2	
( <i>E</i> )-nerolidyl acetate	1717	0.4 $\pm$ 0.0			
( <i>Z</i> )-nuciferol	1722	3.5 $\pm$ 0.0		0.3 $\pm$ 0.2	
(6 <i>R</i> , 7 <i>R</i> )-bisabolone	1741			3.6 $\pm$ 0.3	
$\gamma$ -costol	1744	7.9 $\pm$ 0.0		3.5 $\pm$ 0.2	1.1 $\pm$ 0.0
cyclocolorenone	1760	1.3 $\pm$ 0.3		0.8 $\pm$ 0.2	
$\gamma$ -curcumen-15-al	1766			0.2 $\pm$ 0.0	
14-hydroxy- $\alpha$ -muurolene	1777			0.5 $\pm$ 0.1	
<b>Total identified</b>		<b>98.9</b>	<b>99.0</b>	<b>95.4</b>	<b>99.8</b>

**Table 2.** Quantity and types of compounds identified in the essential oils

Types of compounds	BM	GB	GG	UG
Hydrocarbon monoterpenes			3	
Oxygenated monoterpenes	4	1	1	2
Hydrocarbon sesquiterpenes	18	13	15	7
Oxygenated sesquiterpenes	39	17	39	11
Phenylpropanoids				1
<b>Total identified</b>	<b>61</b>	<b>31</b>	<b>58</b>	<b>21</b>

**Table 3.** Antimicrobial activity of essential oils from the branches of *B. multiflora*, *G. guianensis*, *G. blepharophylla* and *U. guatterioides*

Samples	Minimum inhibition concentration (mg.mL <sup>-1</sup> )					
	SA	EF	SS	PA	EC	SE
<i>Bocageopsis multiflora</i>	0.09	0.05	0.05	1.5	1.5	0.75
<i>Guatteria blepharophylla</i>	0.19	0.19	0.75	3.0	6.0	3.0
<i>G. guianensis</i>	0.09	0.02	0.09	3.0	3.0	3.0
<i>Unonopsis guatterioides</i>	0.09	0.09	0.19	1.5	1.5	0.75

SA = *Staphylococcus aureus* (ATCC 6538), EF = *Enterococcus faecalis* (ATCC 29212), SS = *Streptococcus sanguinis* (ATCC 10556), PA = *Pseudomonas aeruginosa* (ATCC 9027), EC = *Escherichia coli* (ATCC 8739), SE = *Salmonella enterica* (ATCC 13076).

## CONCLUSION

This study of the branches of Annonaceae contributes to the knowledge of the aromatic flora of *terra firme* forest. It also shows the complex mixture of odorous components of the essential oils of the four evaluated species. It was interesting to observe that the terpenes contributed to the bactericidal activity since the essential oil with a high content of the phenylpropanoid elemicin was not very promising for the bacterial strains tested.

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