

Artigo

Chemical Composition and Antimicrobial Activity of Essential Oils from *Xylopia aromatica* (Annonaceae) Flowers and Leaves**Nascimento, M. N. G.; Junqueira, J. G. M.; Terezan, A. P.; Severino, R. P.; Silva, T. S.; Martins, C. H. G.; Severino, V. G. P.****Rev. Virtual Quim.*, 2018, 10 (5), 1578-1590. Data de publicação na Web: 09 de outubro de 2018<http://rvq.sbg.org.br>**Composição Química e Atividade Antimicrobiana dos Óleos Essenciais das Flores e Folhas de *Xylopia aromatica* (Annonaceae)**

Resumo: Os óleos essenciais (OE) das flores e folhas foram obtidos por hidrodestilação e analisados por cromatografia gasosa acoplada à espectrometria de massa (CG-EM). Os principais compostos identificados no OE das flores foram: pentadecan-2-ona (16,38 %), biciclogermacreno (9,74 %), 7-epi- α -eudesmol (7,76 %), khusinol (7,23 %), *n*-tricosano (6,17 %), heptadecan-2-ona (5,83 %), geranyl- α -terpineno (4,46 %) e cedr-8(15)-en-9 α -ol (4,40 %). O OE das folhas apresentou predominantemente espatulenol (27,11 %), khusinol (13,04 %), biciclogermacreno (8,52 %), globulol (6,47 %), *cis*-guaia-3,9-dien-11-ol (5,98 %), 2-epi- α -cedren-3-ona (4,69 %) e elemicina (4,32 %). A atividade antimicrobiana dos óleos essenciais foi testada em cepas bacterianas Gram-positivas e Gram-negativas, além de fungos. As menores concentrações inibitórias mínimas (CIM) foram observadas frente à *Streptococcus pyogenes* ATCC 12345 (200 e 100 $\mu\text{g mL}^{-1}$) para as flores e folhas, respectivamente. Quanto ao potencial antifúngico, o OE das folhas apresentou atividade moderada (500 $\mu\text{g mL}^{-1}$) frente à *Candida albicans*. Em suma, este estudo reporta dados sobre a composição química e atividade antimicrobiana do OE de flores *X. aromatica*, contribuindo para o conhecimento sobre esta espécie

Palavras-chave: *Xylopia aromatica*; flores; óleo essencial; CG-EM; atividade antimicrobiana.

Abstract

The essential oils (EO) from flowers and leaves were obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry. The main compounds found in the flower oil were pentadecan-2-one (16.38 %), bicyclogermacrene (9.74 %), 7-epi- α -eudesmol (7.76 %), khusinol (7.23 %), *n*-tricosane (6.17 %), heptadecan-2-one (5.83 %), geranyl- α -terpinene (4.46 %) and cedr-8(15)-en-9 α -ol (4.40 %). The leaf oil contained predominantly spathulenol (27.11 %), khusinol (13.04 %), bicyclogermacrene (8.52 %), globulol (6.47 %), *cis*-guaia-3,9-dien-11-ol (5.98 %), 2-epi- α -cedren-3-one (4.69 %) and elemicin (4.32 %). The antimicrobial activity of the EO was tested against Gram-positive and Gram-negative bacterial strains and fungi. The flower and leaf oils exhibited the lowest minimum inhibitory concentrations (MIC) against *Streptococcus pyogenes* ATCC 12345 (200 and 100 $\mu\text{g mL}^{-1}$ respectively). As for antifungal potential, the EO from leaves showed moderate activity (500 $\mu\text{g mL}^{-1}$) against *Candida albicans*. This study offers the first report about the chemical composition and antimicrobial activity of the EO from *X. aromatica* flowers and contributes to the body of knowledge about this species.

Keywords: *Xylopia aromatica*; flowers, essential oil; GC-MS; antimicrobial activity.

* Universidade Federal de Goiás, Instituto de Química, Regional Goiânia, Campus Samambaia, CEP 74690-900, Goiânia-GO, Brasil.

✉ yanessa.pasqualotto@pq.cnpq.br

DOI: [10.21577/1984-6835.20180107](https://doi.org/10.21577/1984-6835.20180107)

Chemical Composition and Antimicrobial Activity of Essential Oils from *Xylopia aromatica* (Annonaceae) Flowers and Leaves

Michelle N. G. do Nascimento,^a João Gabriel M. Junqueira,^a Ana Paula Terezan,^a Richele Priscila Severino,^a Thayná de Souza Silva,^b Carlos Henrique G. Martins,^b Vanessa G. P. Severino^c

^a Federal University of Catalão, Department of Chemistry, Lamartine Pinto de Avelar avenue 1120, CEP 75704-020, Catalão, Brazil.

^b Franca University, Laboratory of Research and Applied Microbiology, Armando Salles de Oliveira avenue 201, CEP 14404-600 Franca, Brazil.

^c Federal University of Goiás, Institute of Chemistry, Regional Goiânia, Campus Samambaia, Esperança avenue, CEP 74690-900 Goiânia, Brazil

* vanessa.pasqualotto@pq.cnpq.br

Recebido em 16 de julho de 2018. Aceito para publicação em 25 de setembro de 2018

1. Introduction

2. Experimental Section

2.1. Plant material

2.2. Obtaining Essential Oil

2.3. GC-MS Analysis

2.4. Identification of the constituents of essential oils

2.5. Determination of the antimicrobial activity

3. Results and Discussion

3.1. Chemical composition of essential oil from *X. aromatica* flowers and leaves

3.2. Antimicrobial Activity

4. Conclusions

1. Introduction

The genus *Xylopia* is one of the most important ones in the Annonaceae family.¹ It comprises approximately 160 species widely distributed in tropical and subtropical regions of the Americas, Africa, Asia and Oceania. From a chemical point of view, this genus has

been extensively studied because of its numerous odoriferous species.² Recent studies on the evaluation of chemical composition of essential oils (EO) in *Xylopia* species (i.e. *X. frutescens*, *X. sericea*, *X. brasiliensis*, *X. nitida* and *X. langsdorffiana*) have been reported. The major compounds found in the EO from *X. brasiliensis* leaves were spathulenol (40.8 %), 1,8-cineole (11.1

%) and verbenone (11.1 %).³ From the EO of *X. nitida* leaves *p*-cymene (24.6 %) and β -caryophyllene (21.4 %) were the predominant constituents found.⁴ (*E*)-caryophyllene (31.5 %), bicyclogermacrene (15.1 %), germacrene D (9.7 %), σ -cadinene (5.4 %), viridiflorene (5.1 %) and α -copaene (4.4 %) were reported from the leaves of *X. frutescens* with anticancer activity.⁵ The constituents of the EO from the leaves of *X. langsdorffiana* also availed the presence of germacrene D (22.9 %), trans- β -caryophyllene (15.7 %) and α -pinene (7.3 %) with molluscicidal activity.⁶ Finally, in the EO composition of *X. sericea* fruits there were cubenol (57.4 %) and α -epi-muurolool (26.1 %), which presented acaricidal activity.⁷

Xylopia aromatica (Lam.) Mart., popularly known as “pimenta-de-macaco,” is a small tree (4-5 m tall) commonly occurring in Brazil’s coastal forests and savannas (Cerrado).⁸ This plant is widely cultivated because of its attractively scented white flowers and its use in folk medicine as a carminative, stimulant, diuretic, treatment of digestive diseases and spice for seasoning meat.^{9,10} The leaves, fruit and stem bark have already been studied by analyzing their essential oils (EO).^{2,9,11} However, the constituents of the EO of *X. aromatica* flowers have not yet been determined, and so far only the floral scent has been examined.^{12,13}

With respect to its biological action, the insecticidal activity and antiseptic and analgesic properties of various *Xylopia* spp. extracts have been reported.⁸ The bacteriostatic and fungistatic activities of EO from the stem bark and leaves of *X. aromatica* against some microorganisms have also been determined.¹⁰ However, to date, no information is available about the antimicrobial activity of the EO of its flowers.

In this work, a variety of microorganisms that cause skin disorders were selected to evaluate the antimicrobial activity of the aromatic essential oil of *X. aromatica*. These microorganisms include *Staphylococcus aureus*, *Streptococcus pyogenes* and *Propionibacterium acnes*, which are Gram-positive bacteria that can cause disorders ranging from pharyngitis and mild skin

infections (such as impetigo) to serious infections such as scarlet fever¹⁴ and infective endocarditis, resulting in considerable global human morbidity and mortality.¹⁵

Pseudomonas aeruginosa and *Burkholderia cepacia* are Gram-negative bacteria associated with infections that include pneumonia, bacteremia, skin and soft tissue infection and genitourinary tract infection.^{16,17} Almost 10 % of all hospital-acquired infections are caused by *P. aeruginosa*, which kills thousands of people every year.¹⁸

Candida species are opportunistic pathogens that have been considered the second most frequent cause of fungal infections in humans worldwide.^{19,20} To date, there are no vaccines against any fungal pathogens. Only a few antifungal agents are in clinical use and therapies are limited by drug-safety considerations.^{20,21}

This paper offers the first report of the composition and antimicrobial effect of EO of *X. aromatica* flowers. Previous studies have investigated the antimicrobial activity of EO of *X. aromatica* leaves against *S. aureus*, *Mycobacterium smegmatis*, *Escherichia coli*, *P. aeruginosa* and *C. albicans*;¹⁰ here, we report the activity of this EO against *B. cepacia*, *S. pyogenes*, *P. acnes* and *C. tropicalis* for the first time. In addition, the chemical composition of the leaf oil is analyzed and compared with results of previous studies.

2. Experimental Section

2.1. Plant material

X. aromatica flowers and leaves were collected in the mornings, the former in October 2014 and the latter in February 2015, at the Federal University of Goiás in Catalão city. The leaves were collected randomly, while the flowers selected were those with open corolla (both obtained from fully developed specimens). The species was identified and a voucher was deposited at the

Centro-Norte-Matogrossense Herbarium of the Federal University of Mato Grosso – UFMT – Campus Sinop, under number 6554.

2.2. Obtaining essential oil

Fresh *X. aromatica* flowers and leaves (300 g each) were subjected to hydrodistillation for 4 h in a Clevenger-type water steam distillation apparatus. The oil thus collected was dried over anhydrous sodium sulfate, resulting in strong-smelling pale-yellow oil in yields of 0.2 and 0.1 % (w/w) from flowers and leaves, respectively, that was stored at 7 °C until analysis. All the experiments were carried out in triplicate.

2.3. GC-MS analysis

The EO from flowers was analyzed in a gas chromatograph (Agilent Technologies, 7820 A) coupled to a mass spectrometer (MSD 5975, Agilent Technologies, USA), equipped with a non-polar HP-5 MS fused silica capillary column (30m x 0.250µm, 0.25µm film thickness; Agilent). The EO from leaves was analyzed in a gas chromatograph coupled to a mass spectrometer (Shimadzu GC-MS QP5000), equipped with a DB-5 MS capillary column (30m x 0.250µm, 0.25µm film thickness). The injector, interface, ionization source and quadrupole temperatures were 250, 280, 230 and 150 °C, respectively, in the two devices. Helium was used as the carrier gas at a flow rate of 1 mL min⁻¹ and a split ratio of 10:1. The injection volume was 1µL of diluted solution (1/1000) of oils in *n*-hexane. The column temperature was set from 60 °C (2 min) to 250 °C at a heating rate of 4 °C min⁻¹; 250 °C (10.5 min). MS spectra were obtained using electron impact at 70 eV, a scan interval of 3.46s, and fragments from 45 to 450 u.m.a.

2.4. Identification of the Constituents of Essential Oils

The chemical constituents were identified based on the Kovats index (KI), which was determined in relation to a homologous series of *n*-alkanes (C₈-C₃₀) run under the same operating conditions, as well as comparisons with authentic compounds. The KI obtained was compared with KI of the NIST Standard Reference Data²² and the Adams book.²³ In addition, comparison of the mass spectra and their similarity index to those stored in the libraries was applied (Nist08, Wiley139, Wiley 229, ShimDemo, and Shim2205 libraries) or to mass spectra reported in the literature.^{22,23}

2.5. Determination of the Antimicrobial Activity

The antimicrobial activity of the EO samples from *X. aromatica* flowers and leaves was evaluated against: a) five strains of Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300, *Streptococcus pyogenes* ATCC 12345 and *Propionibacterium acnes* ATCC 11827); b) three strains of Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 14502, *P. aeruginosa* ATCC 27853 and *Burkholderia cepacia* ATCC 17759); and c) two strains of fungi (*Candida albicans* ATCC 4639 and *C. tropicalis* ATCC 13803).

The MIC values were found to be the lowest concentrations that prevented visible growth, using the broth microdilution method in 96-well microplates described previously.^{24,25} The samples were tested at concentrations ranging from 0.195 to 400 µg mL⁻¹. After incubation period, 30 µL of an aqueous solution of 0.02 % resazurin (Sigma-Aldrich) was added to each well. Resazurin is an oxidation reduction probe that allows for immediate observation of microbial growth. The blue and red colors represent the absence and presence of microbial growth, respectively.²⁶

3. Results and Discussion

3.1. Chemical Composition of Essential Oil from *X. aromatica* Flowers and Leaves

The EOs of *X. aromatica* were obtained by hydrodistillation in yields of 0.2 and 0.1 % (w/w) from flowers and leaves, respectively. The results of this chemical analysis are presented in Tables 1 and 2. The twenty-eight constituents identified in the oils from flowers are listed in Table 1. Sesquiterpenes represented the main class (68.94 %) of

detected compounds. The main sesquiterpenes were bicyclogermacrene (9.74 %), zonarene (2.90 %) and δ -cadinene (2.40 %), while 7-epi- α -eudesmol (7.76 %), khusinol (7.23 %) and cedr-8(15)-en-9 α -ol (4.40 %) were the main oxygenated sesquiterpenes. However, the major constituent identified in this oil was pentadecan-2-one (16.38 %). Ketones correspond to 10.34 % of volatile crude oil. Among other compounds, considerable amounts of *n*-tricosane (6.17 %), heptadecan-2-one (5.83 %), geranyl- α -terpinene (4.46 %), cubenol (3.44 %), globulol (3.13 %) and spathulenol (3.00 %) were detected.

Table 1. Chemical constituents of the essential oil from *X. aromatica* flowers

R _t (min)	Compound ¹	KI		Area (%)	Criteria ³
		Calculated ²	Literature		
15.81	neral	1245	1238	1.51	a, c
18.93	δ -elemene	1340	1338	0.76	a, b, c
23.49	germacrene D	1485	1485	1.33	a, b, c
23.97	bicyclogermacrene	1501	1500	9.74	a, b, c
24.54	δ -cadinene	1520	1523	2.40	a, b, c
24.76	zonarene	1527	1529	2.90	a, b, c
26.38	spathulenol	1581	1578	3.00	a, b, c
26.56	globulol	1587	1590	3.13	a, b, c
26.79	viridiflorol	1595	1592	1.93	a, b, c
26.96	guaiol	1600	1600	1.36	a, b, c
27.10	5-epi-7-epi- α -eudesmol	1605	1607	0.90	a, c
27.23	β -atlantol	1610	1608	1.70	a, b, c
27.41	isolongifolan-7- α -ol	1617	1619	1.14	a, c
27.61	unknown 1	1624	-	2.32	-
27.82	<i>cis</i> -cadin-4-en-7-ol	1632	1636	2.52	a, c
28.22	cubenol	1646	1646	3.44	a, b, c
28.28	cedr-8(15)-en-9 α -ol	1649	1651	4.40	a, c
28.46	α -eudesmol	1655	1653	1.68	a, b, c
28.56	7-epi- α -eudesmol	1659	1663	7.76	a, b, c
28.80	ledene oxide II	1668	-	1.88	b, c

R _t (min)	Compound ¹	KI		Area (%)	Criteria ³
		Calculated ²	Literature		
29.01	khusinol	1675	1680	7.23	a, b, c
29.72	pentadecan-2-one	1702	1697	16.38	a, b, c, d
30.38	geranyl- α -terpinene	1726	-	4.46	b, c
30.78	eremophilone	1740	1736	0.60	a, b, c
34.98	heptadecan-2-one	1904	1906	5.83	a, b, c, e
38.73	manool	2060	2057	0.77	a, b, c
39.63	<i>n</i> -heneicosane	2100	2100	2.92	a, b, c
43.98	<i>n</i> -tricosane	2299	2300	6.17	a, b, c
47.99	<i>n</i> -pentacosane	2499	2500	1.10	a, b, c
Classes of compounds (%)					
<i>Oxygenated monoterpenes</i>					
Aldehydes					3.44
<i>Sesquiterpene hydrocarbons</i>					17.24
<i>Oxygenated sesquiterpenes</i>					
Alcohols					44.82
Ketones					3.44
Epoxides					3.44
<i>Diterpene hydrocarbons</i>					3.44
<i>Oxygenated diterpene</i>					3.44
<i>Straight chain aliphatic compounds</i>					
Hydrocarbons					10.34
Ketones					6.89
<i>Unknown</i>					3.44
Total of all compounds					99.93
m/z (Rel. Int.): unknown 1: 218(100), 175(98), 147(50), 91(48), 105(40).					

¹Compound listed in order of elution. ²Kovats index on an HP-5 MS column, determined experimentally using a homologous series of *n*-alkanes. ³Compound identification criteria: (a) calculated KI compared with KI from the Adams book;²³ (b) comparison of MS with those of database (Shim, Wiley, NIST08, NIST08s libraries and NIST Standard Reference Data;²² (c) comparison of MS;²³ (d) comparison with commercial standard; (e) Souza *et al.* 2009.³⁴

The forty-seven constituents identified from leaves are listed in Table 2, corresponding 95.12 % of the total oil. Oxygenated sesquiterpenes were the main constituents (71.25 % of the EO). The main representatives of this class were spathulenol

(27.11 %), khusinol (13.04 %), globulol (6.47 %), *cis*-guaia-3,9-dien-11-ol (5.98 %) and 2-epi- α -cedren-3-one (4.69 %). Sesquiterpene hydrocarbons represented 9.62 % of the detected compounds and bicyclogermacrene (8.52 %) was the major constituent of this class. Among other compounds, small amounts of elemicin (4.32 %), viridiflorol (2.51 %), muurolo-4-10(14)-dien-1- β -ol (2.39 %), (*Z*)-14-hydroxy-caryophyllene (2.35 %) and geraniol acetate (2.24 %) were detected.

By comparison with previously extracted EO, our analyses confirmed the data reported, where the main compound was 27.5 % and 64.4 % spathulenol in species from Bolivia¹⁰

and Cuba,²⁷ respectively. On the other hand, the main compound in the species occurring in North Brazil⁹ was sesquiterpene bicyclogermacrene (36.5 %), followed by spathulenol (20.5 %). However, a much smaller amount of spathulenol (3.1 %) was found in the species occurring in Southeast Brazil,¹¹ and α -pinene (26.1 %) was the major compound, followed by limonene (22.3 %), and bicyclogermacrene (20.4 %). It is interesting to note that bicyclogermacrene sesquiterpene is the precursor of spathulenol, an oxygenated sesquiterpene that can be formed by enzymatic oxidation of bicyclogermacrene.²⁸

Table 2. Chemical constituents of the essential oil from *X. aromatica* leaves

R _t (min)	Compound ¹	KI		Area (%)	Criteria ³
		Calculated ²	Literature		
14.95	α -terpineol	1194	1188	0.47	a, b, c
15.81	<i>trans</i> -carveol	1219	1216	0.18	a, b, c
16.92	geraniol	1250	1252	1.73	a, b, c
19.33	citronellic acid	1319	1313	0.23	a, b, c
19.40	methyl geranate	1321	1324	0.13	a, b, c
19.81	δ -elemene	1332	1338	0.16	a
20.38	citronellyl acetate	1349	1352	0.17	a, b
21.37	geraniol acetate	1377	1381	2.24	a, b, c
21.47	geranic acid	1380	1375	0.08	b, c, e
21.79	β -elemene	1389	1390	tr	a, d
23.43	aromadendrene	1439	1441	0.09	a
24.06	alloaromadendrene	1459	1460	0.05	a, d
24.71	(<i>E</i>)- β -ionone	1479	1488	0.10	a, b
24.82	germacrene D	1483	1485	0.46	a, b, c
25.15	viridiflorene	1493	1496	0.08	a, b, c
25.33	bicyclogermacrene	1499	1500	8.52	a, b
25.54	<i>trans</i> - β -guaiene	1505	1502	0.17	a
25.96	cubebol	1518	1515	0.09	a
25.98	δ -cadinene	1519	1523	0.09	a, b
26.90	elemicin	1548	1557	4.32	a, b

27.41	(<i>E</i>)-nerolidol	1564	1563	0.73	a
27.70	(3 <i>Z</i>)-hexenyl benzoate	1572	1566	0.93	a, b
28.02	spathulenol	1583	1578	27.11	a, b, c
28.25	globulol	1590	1590	6.47	a, b, c
28.48	viridiflorol	1597	1592	2.51	a, b, c
28.76	humulene epoxide II	1606	1608	0.43	a
29.29	2-epi- α -cedren-3-one	1625	1627	4.69	a
29.44	1-epi-cubenol	1630	1628	1.23	a
29.60	muurola-4-10(14)-dien-1- β -ol	1636	1631	2.39	a
30.06	<i>cis</i> -guaia-3,9-dien-11-ol	1652	1649	5.98	a
30.29	(<i>Z</i>)-14-hydroxy-caryophyllene	1660	1667	2.35	a
30.60	unknown 1	1671	-	2.84	-
30.92	khusinol	1682	1680	13.04	a
31.32	germacra-4(15),5,10(14)-trien-1- α -ol	1696	1686	0.61	a, b, c
31.50	cyperotundone	1702	1695	1.71	a, c
31.75	14-hydroxy-4,5-dihydro-caryophyllene	1711	1706	0.28	a, b
32.47	isobicyclogermacrenal	1736	1734	1.32	a, b, c
33.28	4,14-anhydro-amorpha-4,9-diene	1764	1756	0.16	a
35.40	6,10,14-trimethyl-2-pentadecanone	1841	1842	0.10	b, c, f
36.58	3,4-benzocinnoline	1886	-	0.32	g
36.98	<i>n</i> -nonadecane	1901	1900	0.10	a
37.56	carissone	1923	1927	0.15	a
38.61	caprylic ether	1963	-	0.77	b, c
39.38	1-eicosene	1993	1988	0.17	a, b
40.95	manool	2057	2057	0.96	a, b, c
42.25	phytol	2111	2111	0.87	b, c, h
42.91	unknown 2	2138	-	0.24	-
44.20	1-docosene	2192	2189	0.14	a, b, c
Classes of compounds (%)					
<i>Oxygenated monoterpenes</i>					

alcohols	2.38
acid + acetate ester	0.36
Sesquiterpene hydrocarbons	9.62
Oxygenated sesquiterpenes	
Alcohols	62.79
aldehydes	1.32
ketones	6.55
epoxides	0.59
Oxygenated diterpenes	
alcohols	1.20
Straight chain aliphatic compounds	
hydrocarbons	0.41
alcohols	0.87
ketones	0.20
acids + ether	7.58
benzenoids	1.25
Unknown	2.84
Total of all compounds	97.96
m/z (Rel. Int.): unknown 1: 177(100), 123(98), 55(73), 159(69), 91(59). unknown 2: 55 (100), 81 (83), 93 (68), 107 (48), 123 (30).	

¹Compound listed in order of elution. ²Kovats Index on DB-5MS column, determined experimentally using a homologous series of *n*-alkanes. ³Compound identification criteria: (a) calculated KI compared with KI from the Adams book;²³ (b) comparison of MS with those of database (Shim, Wiley, NIST08, NIST08s libraries and NIST Standard Reference Data;²² (c) similarity index with mass spectral database (Shim, Wiley, and Nist Libraries); (d) comparison with commercial standard; (e) Ouamba *et al.* 2006;³⁵ (f) Smelcerovic *et al.* 2007;³⁶ (g) Rostad *et al.* 1986;³⁷ (h) Kucic *et al.* 2006.³⁸

As for the contribution to the aromatic plant fingerprint, it should be noted that the volatile compounds in different parts of *X. aromatica* differ. Only δ -elemene, germacrene D, bicyclogermacrene, δ -cadinene, spathulenol, globulol, viridiflorol, khusinol, and manool were found in both oils. The latter compound, the major one in flower oil (16.38%), was not found in the leaf oil. On the other hand, spathulenol, the main compound in leaf oil (27.11%), was present as 3.00% of the flower oil. This difference can be

partly explained by the existence of distinct secretory structures in different plant parts.²⁹

3.2. Antimicrobial activity

The MIC results are listed in Table 3. With regard to the antimicrobial activity of the plant material, MIC values of less than or equal to 100 $\mu\text{g mL}^{-1}$ were characterized as good activity; from 100 to 500 $\mu\text{g mL}^{-1}$ as moderate; from 500 to 1000 $\mu\text{g mL}^{-1}$ as weak;

and over 1000 $\mu\text{g mL}^{-1}$ as inactive against both Gram-positive and Gram-negative bacteria and yeasts.³⁰ In this study, samples displaying a MIC of over 400 $\mu\text{g mL}^{-1}$ against bacteria and of over 2000 $\mu\text{g mL}^{-1}$ for yeast were considered inactive.

The flower oil was found to have an inhibitory bacterial effect against *S. pyogenes* (ATCC 12345) (MIC 200 $\mu\text{g mL}^{-1}$), *S. aureus*

(ATCC 29213, 25923 and 43300) (MIC 400 $\mu\text{g mL}^{-1}$) and *P. aeruginosa* strains (ATCC 27853 and 14502) (MIC 400 $\mu\text{g mL}^{-1}$). However, no activity against *B. cepacia* ATCC 17759 and *P. acnes* ATCC 11827 (MIC > 400 $\mu\text{g mL}^{-1}$) was detected. As for its antifungal potential, this oil presented weak activity (MIC 2000 $\mu\text{g mL}^{-1}$) against *Candida* strains (ATCC 4639 and 13803).

Table 3. Antimicrobial activity of the essential oils of *X. aromatica* flowers and leaves

Microorganism	MIC ($\mu\text{g mL}^{-1}$)		
	Essential Oil		Reference Drug
	Flowers	Leaves	
<i>Burkholderia cepacia</i> ATCC 17759	>400	>400	5.9 ^c
<i>Pseudomonas aeruginosa</i> ATCC 14502	400	400	5.9 ^c
<i>Pseudomonas aeruginosa</i> ATCC 27853	400	400	> 5.9 ^c
<i>Staphylococcus aureus</i> ATCC 25923	400	400	0.37 ^d
<i>Staphylococcus aureus</i> ATCC 43300	400	400	2.95 ^d
<i>Staphylococcus aureus</i> ATCC 29213	400	400	0.74 ^d
<i>Streptococcus pyogenes</i> ATCC 12345	200	100	0.37 ^d
<i>Candida albicans</i> ATCC 4639	2000	500	nt
<i>Candida tropicalis</i> ATCC 13803	2000	2000	nt
<i>Candida krusei</i> ATCC 6258 ^a	nt	nt	2.0 ^e
<i>Candida parapsilosis</i> ATCC 22019 ^a	nt	nt	1.0 ^e
<i>Propionibacterium acnes</i> ATCC 11827	>400	>400	nt
<i>Bacteroides fragilis</i> ATCC 25285 ^a	nt	nt	1.47 ^b
<i>Bacteroides thetaiotaomicron</i> ATCC 29741 ^a	nt	nt	2.95 ^b

^acontrol strains; ^bmetronidazole; ^cstreptomycin; ^dpenicillin; ^eamphotericin; nt: not tested

The leaf oil showed good activity (100 $\mu\text{g mL}^{-1}$) against *S. pyogenes* (ATCC 12345). For other bacteria, the MIC values were moderate (400 $\mu\text{g mL}^{-1}$), and no activity was detected against *B. cepacia* ATCC 17759 and *P. acnes* ATCC 11827 (MIC > 400 $\mu\text{g mL}^{-1}$). Although previous studies have reported MIC values of 20 mg mL^{-1} against *S. aureus* and *C. albicans*, and no activity against *P. aeruginosa* (MIC > 20 mg mL^{-1}),¹⁰ in this study we obtained MIC

values of 400 $\mu\text{g mL}^{-1}$ against *S. aureus* and *P. aeruginosa*, and 500 $\mu\text{g mL}^{-1}$ against *C. albicans*. Although spathulenol is present in similar proportions in *X. aromatica* leaf oils from Brazil, studied here, and Bolivia,¹⁰ the other identified components differ widely. This may explain the different MIC values found in the two cases.

The significant antimicrobial activity of the EO from *X. aromatica* leaves can probably be

explained by the fact that the leaves contain a larger amount of oxygenated sesquiterpenes (71.25 %) than the flowers (51.70 %). This finding has been reported in previous studies, which demonstrated that the presence of an oxygenated function in terpene enhances its antibacterial activity.^{31, 32}

In addition, some studies report that whole EO exhibits better antibacterial activity than its individual components, thus suggesting that the minor compounds are essential to the activity due to the synergistic effect or potentiating influence of the various molecules.³³

4. Conclusion

The analysis of EO from *X. aromatica* revealed significant variations in the chemical composition. Sesquiterpenes represented the main constituents (68.96 % and 80.87 % of flowers and leaves, respectively). Thus, this study offers the first report about the chemical composition and antimicrobial activity of the EO from *X. aromatica* flowers and contributes to the body of knowledge about this species. *In vitro* antimicrobial activity of EOs was evaluated against microorganisms that cause skin lesions and showed moderate activity against *P. aeruginosa*, *S. aureus* and *S. pyogenes*. As for antifungal properties, the leaves showed moderate activity against *C. albicans*. Sesquiterpenes have been reported in the literature by possess antimicrobial activity and this characteristic may be the responsible to the biological activity observed from the EOs. However, further research is needed to draw the association between these compounds and microorganisms evaluated.

Acknowledgements

The authors thank CNPq (grant no. 563286/2010-5) and FAPEG (grant no. 200910267000366) for their financial support.

References

- Moreira, I. C.; Roque, N. F.; Vilegas, W.; Zalewski, C. A.; Lago, J. H. G.; Funasaki, M. Genus *Xylopi* (Annonaceae): chemical and biological aspects. *Chemistry and Biodiversity*. **2013**, *10*, 1921. [CrossRef] [PubMed]
- Fournier, G.; Leboeuf, M.; Cavé, A. Annonaceae Essential Oils: A Review. *Journal of Essential Oil Research* **1999**, *11*, 131. [CrossRef]
- Lago, J. H. G.; Moreira, I. C.; Tanizaki, T. M.; Moreno, P. R. H.; Roque, N. F.; Limberger, R. P.; Apel, M. A.; Henriques, A. T. Mono and sesquiterpenes from the leaf essential oil of *Xylopi* *brasiliensis* Spreng. (Annonaceae). *Journal of essential oil research* **2003**, *15*, 406. [CrossRef]
- Fournier, G.; Hadjiakhoondi, A.; Charles, B.; Leboeuf, M.; Cavé, A. Volatile constituents of *Xylopi* *nitida* leaf oil. *Planta Medica*. **1993**, *59*, 185. [CrossRef]
- Ferraz, R. P. C.; Cardoso, G. M. B.; Silva, T. B.; Fontes, J. E. N.; Prata, A. N.; Carvalho, A.; Moraes, M. O.; Pessoa, C.; Costa, E.V.; Bezerra, D. P. Antitumour properties of the leaf essential oil of *Xylopi* *frutescens* Aubl. (Annonaceae). *Food Chemistry* **2013**, *141*, 196. [CrossRef] [PubMed]
- Tavares, J. F.; Silva, M. V. B.; Queiroga, K. F.; Martins, R. M.; Silva, T. M. S.; Camara, C. A.; Agra, M. F.; Barbosa-Filho, J. M.; Silva, M. S.; Marques, M. O. M. Composition and molluscicidal properties of essential oils from leaves of *Xylopi* *langsдорffiana* A. St. Hil. Et Tul. (Annonaceae). *Journal of essential oil research* **2007**, *19*, 282. [CrossRef]
- Pontes, W. J. T.; Oliveira, J. C. S.; Câmara, C. A. G.; Gondim Jr., M. G. C.; Oliveira, J. V. Schwartz, M. O. E. Atividade acaricida dos óleos essenciais de folhas e frutos de *Xylopi* *sericea* sobre o ácaro rajado (*Tetranychus urticae* KOCH). *Química. Nova* **2007**, *30*, 838. [CrossRef]
- Stashenko, E. E.; Jaramillo, B. E.; Martínez, J. R. Analysis of volatile secondary metabolites from Colombian *Xylopi* *aromatica* (Lamarck) by different extraction and headspace

- methods and gas chromatography. *Journal of Chromatography A* **2004**, 1025, 105. [CrossRef] [PubMed]
- ⁹ Maia, J. G. S.; Andrade, E. H. A.; Silva, A. C. M.; Oliveira, J.; Carreira, L. M. M.; Araújo, J. S. Leaf volatile oils from four Brazilian *Xylopi* species. *Flavour and Fragrance Journal* **2005**, 20, 474. [CrossRef]
- ¹⁰ Fournier, G.; Hadjiakhoondi, A.; Charles, B.; Fourniat, J.; Leboeuf, M.; Cavé, A. Chemical and biological studies of *Xylopi aromatica* stem bark and leaf oils. *Planta Medica* **1994**, 60, 283. [CrossRef] [PubMed]
- ¹¹ Lago, J. H. G.; Ávila Jr, P.; Moreno, P. R. H.; Limberger, R. P.; Apel, M. A.; Henriques, A. T. Analysis, comparison and variation on the chemical composition from the leaf volatile oil of *Xylopi aromatica* (Annonaceae). *Biochemical Systematics and Ecology*. **2003b**, 31, 669. [CrossRef]
- ¹² Jürgens, A.; Webber, A. C.; Gottsberger, G. Floral scent compounds of Amazonian Annonaceae species pollinated by small beetles and thrips. *Phytochemistry* **2000**, 55, 551. [CrossRef] [PubMed]
- ¹³ Andrade, E. H. A.; Silva, A. C. M.; Carreira, L. M. M.; Oliveira, J.; Maia, J. G. S. Essential oil composition from leaf, fruit and flower of *Xylopi aromatica* (Lam.) Mart. *Journal Essential Oil Bearing Plants* **2004**, 7, 151. [CrossRef]
- ¹⁴ Walker, M. J.; Barnett, T. C.; McArthur, J. D.; Cole, J. N.; Gillen, C. M.; Henningham, A.; Sriprakash, K. S.; Sanderson-Smith, M. L.; Nizet, V. Disease manifestations and pathogenic mechanisms of group A *Streptococcus*. *Clinical Microbiology Reviews*. **2014**, 27, 264. [CrossRef] [PubMed]
- ¹⁵ Franklin, D.; Lowy, M. D. *Staphylococcus aureus* infections. *The New England Journal of Medicine* **1998**, 339, 520. [CrossRef] [PubMed]
- ¹⁶ Omar, N.; H. Raouf, A. E.; Okasha, H.; Nabil, N. Microbiological assessment of *Burkholderia cepacia* complex (Bcc) isolates in Alexandria Main University Hospital. *Alexandria Journal of Medicine* **2015**, 51, 41. [CrossRef]
- ¹⁷ Soheili, V.; Bazzaz, B. S. F.; Abdollahpour, N.; Hadizadeh, F. Investigation of *Pseudomonas aeruginosa* quorum-sensing signaling system for identifying multiple inhibitors using molecular docking and structural analysis methodology. *Microbial Pathogenesis* **2015**, 89, 73. [CrossRef] [PubMed]
- ¹⁸ Zou, Y.; Nair, S. K. Molecular basis for the recognition of structurally distinct autoinducer mimics by the *Pseudomonas aeruginosa* LasR Quorum sensing signaling receptor. *Chemistry & Biology*. **2009**, 16, 961. [CrossRef] [PubMed]
- ¹⁹ Castro, R. D.; Lima, E. O. Anti-candida activity and chemical composition of *Cinnamomum zeylanicum* blume essential oil. *Brazilian Archives of Biology and Technology*. **2013**, 56, 749. [CrossRef]
- ²⁰ Whibley, N.; Gaffen, S. L. Beyond *Candida albicans*: mechanism of immunity to non-albicans *Candida* species. *Cytokine* **2015**, 76, 42. [CrossRef] [PubMed]
- ²¹ Quiroga, E. D.; Cormick, M. P.; Pons, P.; Alvarez, M. G.; Durantini, E. N. Mechanistic aspects of the photodynamic inactivation of *Candida albicans* induced by cationic porphyrin derivatives. *European Journal of Medicinal Chemistry* **2012**, 58, 332. [CrossRef] [PubMed]
- ²² Data NIST Standard Reference. Available online: <<http://webbook.nist.gov/chemistry/name-ser.htm>>. (accessed on 15th November 2017).
- ²³ Adams, R. P.; *Identification of essential oil components by Gas Chromatography/Mass Spectrometry*, Allured Publishing Corporation: Carol Stream, 2007.
- ²⁴ Moraes, T. S.; Leandro, L. F.; Silva, L. O.; Santiago, M. B.; Souza, A. B.; Furtado, R. A.; Tavares, D. C.; Veneziani, R. C.; Ambrósio, S. R.; Bastos, J. K.; Martins, C. H. In vitro evaluation of *Copaifera oblongifolia* oleoresin against bacteria causing oral infections and assessment of its cytotoxic potential. *Current Pharmaceutical Biotechnology* **2016**, 17, 894. [CrossRef] [PubMed]
- ²⁵ Souza, C. M.; Pereira Jr., S. A.; Moraes, T. S.; Damasceno, J. L.; Amorim, M. S.; Dias, H. J.;

- Stefani, R.; Tavares, D. C.; Martins, C. H.; Crotti, A. E.; Mendes-Giannini, M. J.; Pires, R. H. Antifungal activity of plant-derived essential oils on *Candida tropicalis* planktonic and biofilms cells. *Medical Mycology* **2016**, *54*, 515. [[CrossRef](#)] [[PubMed](#)]
- ²⁶ Sarker, S. D.; Nahar, L.; Kumarasamy, Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods* **2007**, *42*, 321. [[CrossRef](#)] [[PubMed](#)]
- ²⁷ Pino, J. A. Bello, A.; Urquiola, A.; Garcia, S.; Rosado, A. Leaf oil of *Xylopia aromatica* (Lam.) Mart. from Cuba. *Journal of Essential Oil Research* **2000**, *12*, 751. [[CrossRef](#)]
- ²⁸ Bülow, N.; König, W. A. The role of germacrene D as a precursor in sesquiterpene biosynthesis: investigations of acid catalyzed, photochemically and thermally induced rearrangements. *Phytochemistry* **2000**, *55*, 141. [[CrossRef](#)] [[PubMed](#)]
- ²⁹ Figueiredo, A. C.; Barroso, J. G.; Pedro, L. G.; Scheffer, J. C. Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour and Fragrance Journal* **2008**, *23*, 213. [[CrossRef](#)]
- ³⁰ Bardají, D. K. R.; Reis, E. B.; Medeiros, T. C. T.; Lucarini, R.; Crotti, A. E. M.; Martins, C. H. G. Antibacterial activity of commercially available plant-derived essential oils against oral pathogenic bacteria. *Natural Product Research* **2016**, *30*, 1178. [[CrossRef](#)] [[PubMed](#)]
- ³¹ Naigre, R.; Kalck, P.; Roques, C.; Roux, I.; Michel, G. Comparison of antimicrobial properties of monoterpenes and their carbonylated products. *Planta Medica* **1996**, *62*, 275. [[CrossRef](#)] [[PubMed](#)]
- ³² Dorman, H. J. D.; Deans, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology* **2000**, *88*, 308. [[CrossRef](#)] [[PubMed](#)]
- ³³ Burt, S. Essential oils: their antibacterial properties and potential applications in foods - a review. *International Journal of Food Microbiology* **2004**, *94*, 223. [[CrossRef](#)] [[PubMed](#)]
- ³⁴ Souza, P. P. Cardeal, Z. L.; Augusti, R.; Morrison, P.; Marriott, P. J. Determination of volatile compounds in Brazilian distilled cachaça by using comprehensive two-dimensional gas chromatography and effects of production pathways. *Journal of Chromatography A* **2009**, *1216*, 2881. [[CrossRef](#)] [[PubMed](#)]
- ³⁵ Ouamba, J. M.; Ouabonzi, A.; Ekouya, A.; Bessièrè, J. M.; Menut, C.; Abena, A. A.; Banzouzi, J. T. Volatile constituents of the essential oil leaf of *Lantana salvifolia* Jacq. (Verbenaceae). *Flavour and Fragrance Journal* **2006**, *21*, 158. [[CrossRef](#)]
- ³⁶ Smelcerovic, A.; Spiteller, M.; Ligon, A. P.; Smelcerovic, Z.; Raabe, N. Essential oil composition of *Hypericum* L. species from Southeastern Serbia and their chemotaxonomy. *Biochemical Systematics and Ecology* **2007**, *35*, 99. [[CrossRef](#)]
- ³⁷ Rostad, C. E.; Pereira, W. E. Kovats and Lee retention indices determined by gas chromatography/mass spectrometry for organic compounds of environmental interest. *Journal of High Resolution Chromatography & Chromatography Communications* **1986**, *9*, 328. [[CrossRef](#)]
- ³⁸ Kukić, J.; Petrović, S.; Pavlović, M.; Couladis, M.; Tzakou, O.; Niketić, M. Composition of essential oil of *Stachys alpina* L. ssp. *Dinarica* Murb. *Flavour and Fragrance Journal* **2006**, *21*, 539. [[CrossRef](#)]