SUPPLEMENTARY MATERIAL

Chemical composition and antimicrobial activity of the essential oils of *Onychopetalum amazonicum* R.E.Fr.

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The essential oils from leaves, twigs and trunk bark of *Onychopetalum amazonicum* R.E. Fr. (Annonaceae), obtained by hydrodistillation, were analyzed by GC and GC-MS, and also were evaluated for *in vitro* antimicrobial activity. Forty-one compounds, which correspond to 75.0-92.2% of the oil components, were identified. Major compounds were sesquiterpenes, including (*E*)-caryophyllene, caryophyllene oxide, spathulenol, α -gurjunene, *allo*-aromadendrene, and α -*epi*-cadinol. The oils were evaluated for antimicrobial activities against four bacteria strains and five pathogenic fungi. The oil of the trunk bark exhibited good activity against *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 10538, and *Kocuria rhizophila* ATCC 9341, with minimal inhibitory concentration (MIC) of 62.5 μ g/mL. The essential oil composition and the antimicrobial evaluation are reported for the first time for the genus *Onychopetalum*.

Keywords: Annonaceae; antimicrobial evaluation; *Onychopetalum amazonicum*; essential oil.

1. Experimental

1.1. Plant material

The botanical material (leaves, twigs and trunk bark) of *Onychopetalum amazonicum* was collected in March 2015 at the Adolpho Ducke Forest Reserve (26 km along the AM-010 highway, in the city of Manaus, Amazonas state, Brazil) from a specimen previously identified and catalogued (n° 163) by a specialist. A voucher specimen (registration code 218341) was deposited at the herbarium of the Instituto Nacional de Pesquisas da Amazônia (INPA). After collection the plant material was dried at room temperature (ca 26 °C) and pulverized. For the extraction, 100 g of pulverized material (leaves, twigs and trunk bark) were subjected to a hydrodistillation in a Clevenger-type apparatus per four hours. The essential oils were extracted with dichloromethane, dried over anhydrous sodium sulfate and the yield was calculated on the basis of the dry weight of plant. The essential oils were stored in a freezer (-15 °C) prior analysis (Soares et al., 2015).

1.2. GC and GC-MS analysis

Sample oils were directly diluted in dichloromethane to 1 mg/mL for GC and GC-MS analysis. GC analysis was performed on a Shimadzu GC-2010 instrument. Injections (1 µL) at a split ratio of 1:20 were separated on a DB-5 capillary column (30 m, 0.25 mm I.D., 0.25 µm film) with 1.0 mL.min⁻¹. Helium was used as the carrier gas and the injection temperature was 250 °C. The temperature program was a ramp from 60 to 240 °C at 3 °C/min.

A Shimadzu QP2010 apparatus was used for GC-MS analyses with the same column and ramp program. Helium was used as a carrier gas with a flow of 1.0 mL/min. The injection, interface and ion source temperatures were 250, 300 and 200 °C, respectively. Mass spectrometry acquisitions were performed at a mass range of m/z 40-600 with scan velocity of 2 scans/s.

The compound identifications were performed based on comparison of the obtained mass spectra with those stored in the Wiley 8th edition library through GC-MS, and also by comparison of retention index (RI) with literature data (Adam, 2007). RI was calculated according to Van Den Dool and Kratz (1963) equation through the co-injection of a homologous series of linear *n*-alkane (C7-C30).

1.3. Antimicrobial evaluation

The essential oils were evaluated for antimicrobial activity using the broth microdilution method (96-well plates), as previously described (Salvador et al., 2002, Koolen et al., 2013; Silva et al., 2015). Samples were diluted to concentrations between 10.0 and 500.0 µg/mL, and minimal inhibitory concentrations (MIC) were calculated as the lowest concentration showing complete inhibition of a tested strain (Bataglioni et al., 2014). In these tests, chloramphenicol and ketoconazole were used as experimental positive controls for bacteria and fungi strains respectively, while the solution of propylene glycol:sterile distilled water (5:95, v/v) served as diluent (negative control). Antimicrobial activities were detected adding 20 µL of 0.5% triphenyl tetrazolium chloride (TTC, Merck) aqueous solution. MIC values were defined as the lowest concentration of the essential oil that inhibited visible growth, as indicated by TTC staining (dead cells are not stained by TTC). Each sensitivity test was performed in duplicate for each microorganism evaluated and repeated three times. All tested strains of microorganisms are presented in Table S2.

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Captions

Table S1. Leafs, trunk bark and twigs essential oil compositions of *O. amazonicum*.

Table S2. Antimicrobial activity of *O. amazonicum* essential oils estimated by minimum inhibitory concentration (MIC).

Table S1. Leafs, trunk bark and twigs essential oil compositions of *O. amazonicum*.

Compounds	leafs	trunk bark	twigs	RI ^a	RI ^{b,l}	RI ^{b,tb}	RI ^{b,t}
δ-Elemene	0.7	-	-	1335	1338	-	-
α-Cubebene	0.7	1.3	-	1348	1350	1350	-
α-Copaene	8.4	3.4	0.2	1374	1377	1377	1374
β-Bourbonene	0.6	-	-	1387	1386	-	-
β-Elemene	2.7	2.9	4.2	1389	1393	1393	1393
α-Gurjunene	3.6	14.9	10.6	1409	1406	1406	1406
(<i>E</i>)-Caryophyllene	17.0	3.8	-	1417	1421	1421	-
Aromadendrene	1.6	-	-	1439	1440	-	-
α-Humulene	3.1	0.9	-	1452	1455	1455	-
<i>allo</i> -Aromadendrene	-	21.2	2.4	1458	-	1463	1462
γ-Gurjunene	-	1.3	-	1475	-	1476	-
γ-Murolene	2.9	-	-	1478	1478	-	-
Germacrene D	1.7	-	-	1480	1482	-	-
β-Selinene	0.7	0.7	1.4	1489	1487	1487	1487
Bicyclogermacrene	5.4	-	-	1500	1497	-	-
α-Murolene	1.2	0.7	-	1500	1501	1501	-
(<i>E,E</i>)-α-Farnesene	-	-	0.4	1505	-	-	1500
γ-Cadinene	-	1.9	1.8	1513	-	1515	1515
(<i>E</i>)-Calamene	-	-	1.9	1521	-	-	1528
δ-Cadinene	5.9	3.9	2.4	1522	1524	1524	1524
α-Calacorene	2.7	-	1.3	1544	1544	-	1543
Elemol	-	-	2.5	1548	-	-	1550
Germacrene B	1.0	-	-	1559	1558	-	-
β-Calacorene	0.8	-	-	1564	1564	-	-
(<i>E</i>)-Sesquisabinene hydrate	-	-	0.5	1577	-	-	1578
Spathulenol	10.4	-	-	1577	1579	-	-
Caryophyllene oxide	11.9	3.7	4.9	1582	1584	1584	1584
Viridiflorol	0.7	0.9	1.4	1592	1593	1592	1593
Guaiol	1.1	-	-	1600	1598	-	-
Sesquithuriferol	-	-	0.7	1604	-	-	1604
Humulene epoxide II	1.1	-	3.4	1608	1610	-	1608
1,10-di- <i>epi</i> -Cubenol	-	-	3.2	1618	-	-	1615
Isolongifolan-7-α-ol	-	-	2.4	1618	-	-	1621
1- <i>epi</i> -Cubenol	-	3.2	2.8	1627	-	1629	1629
α- <i>epi</i> -Cadinol	-	24.1	14.0	1638	-	1643	1642
Cubenol	1.4	0.8	-	1645	1642	1647	-
α-Cadinol	0.5	1.3	0.7	1652	1655	1655	1651

Cadelene	-	-	0.9	1675	-	-	1675
Mustakone	-	-	1.1	1676	-	-	1678
Eudesma-4(15),7-dien-1 β -ol	-	-	1.8	1687	-	-	1687
Cyperotundone	-	1.3	8.1	1695	-	1695	1695
Sesquiterpene hydrocarbons (%)	60.7	56.9	27.5				
Oxygenated sesquiterpenes (%)	27.1	35.3	47.5				
Total identified (%)	87.8	92.2	75.0				

^aRetention index according to Adams (2007); ^bRetention index on DB5 column calculated according to Van Den Dool and Kratz (1963) for leaf^s, trunk bark^b and twigs^c compounds.

Table S2. Antimicrobial activity of *O. amazonicum* essential oils estimated by minimum inhibitory concentration (MIC).

Microorganism	leaf ^s	trunk bark	twigs	positive controls ^b
	MIC ^a	MIC	MIC	MIC
<i>Staphylococcus aureus</i> (ATCC 14458) ^c	n.a. ^u	n.a	n.a	25.0
<i>Staphylococcus epidermidis</i> (ATCC 12228) ^c	n.a	62.5	n.a	50.0
<i>Escherichia coli</i> (ATCC 10538) ^c	n.a	62.5	n.a	50.0
<i>Kocuria rhizophila</i> (ATCC 9341) ^c	n.a	62.5	n.a	50.0
<i>Candida albicans</i> (ATCC 10231) ^c	n.a	n.a	n.a	12.5
<i>Candida parapsilosis</i> (ATCC 22019) ^c	n.a	n.a	n.a	12.5
<i>Candida tropicalis</i> (ATCC 157) ^c	n.a	n.a	n.a	12.5
<i>Candida glabrata</i> (ATCC 30070) ^c	n.a	n.a	n.a	12.5
<i>Candida dubliniensis</i> (ATCC 778157) ^c	n.a	n.a	n.a	12.5

^aMIC minimum inhibitory concentration in $\mu\text{g/ml}$; ^bPositive control: chloramphenicol for bacteria strains and ketoconazole for yeast strains; ^cstandard strain; ^u n.a = not active, without inhibition of the development. Samples evaluated in the range of 10.0 and 500.0 $\mu\text{g/ml}$.