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Chemical constituents from the leaves of *Annona pickelii* (Annonaceae)

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1. Subject and source

Annona L. belongs to the Annonaceae family and comprises approximately 175 species of trees and shrubs (Chatrou et al., 2009) with the recent inclusion of the genus *Rollinia* (Rainer, 2007; Chatrou et al., 2009; Maas et al., 2011), and is found predominantly in lowland tropical regions. Economically, this genus is the most important of the Annonaceae family due to its edible fruits and medicinal properties (Leboeuf et al., 1982). *Annona pickelii* (Diels) H. Rainer is a small tree (3–5 m tall) endemic to Brazil and popularly known as “araticum-do-mato”, “araticum-da-mata”, “jaquirinha-do-mato”, and “jussara” (Pontes et al., 2004; Maas et al., 2011). It is found only in the States of Paraíba, Pernambuco and Sergipe, occurring in dense forests of “restinga” (Pontes et al., 2004).

The leaves of *A. pickelii* were collected from “Mata do Crasto”, in the city of Santa Luzia do Itanhy [coordinates: 11° 23′ 01″ S, 37° 25′ 13″ W], Sergipe State, Brazil, in March 2010. The identity of the plant was confirmed by Dr. Ana Paula do Nascimento Prata, Department of Biology, Federal University of Sergipe (UFS), Brazil, and a voucher specimen (#15442) has been deposited in the Herbarium, Federal University of Sergipe (ASE/UFS).

2. Previous work

Previous phytochemical studies on this species described the isolation and identification of one lignan known as *epi*-yangambin (De Mesquita et al., 1988) and essential oils (Costa et al., 2011).

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3. Present study

The dried and powdered leaves of *A. pickelii* (363 g) were extracted with petroleum ether (1.5 L, five times), followed by MeOH (1.5 L, five times), yielding extracts of petroleum ether (14.54 g) and MeOH (42.16 g) after removal of each solvent.

A portion of the petroleum ether extract (5.0 g) was subjected to silica gel column chromatography (CC) eluted with increasing concentrations of CH_2Cl_2 in hexane (100:0 to 10:90, v/v), followed by EtOAc in CH_2Cl_2 (100:0 to 10:90, v/v) and MeOH in EtOAc (100:0 to 80:20, v/v), yielding 291 fractions (25 mL each) that were evaluated and pooled according to TLC analysis, resulting in 19 fractions. Fraction 8 (237.8 mg) was subjected to preparative TLC eluted with petroleum ether-EtOAc (90:10, v/v, two times) to yield caryophyllene oxide (**1**, 5.0 mg; Ragasa et al., 2003) and phytol (**3**, 46.5 mg; Arigoni et al., 1999). Fraction 9 (268.6 mg) was further purified on silica gel CC eluted with increasing amounts of EtOAc in petroleum ether (1, 2, 3, 5, 10, 20, 30, 50, 70, 80, 100%, v/v), yielding 55 fractions of 20 mL each that were evaluated and pooled according to TLC analysis, to give eight subfractions. Subfraction 9.2 (160.0 mg) was further purified on preparative TLC eluted with petroleum ether-EtOAc (90:10, v/v, three times) yielding again phytol (**3**, 17.5 mg; Arigoni et al., 1999) and spathulenol (**2**, 43.7 mg; Ragasa et al., 2003). Fraction 10 and 11 were pooled (170.3 mg) and subjected to preparative TLC eluted with petroleum ether-EtOAc (90:10, v/v, three times) over affording phytol (**3**, 29.7 mg; Arigoni et al., 1999) and spathulenol (**2**, 15.5 mg; Ragasa et al., 2003). Fraction 12 (98.6 mg) was further purified on preparative TLC eluted with petroleum ether-EtOAc (80:20, v/v, two times) to yield β-sitosterol (**4**, 22.6 mg; Della Greca et al., 1990). Fraction 17 (989.6 mg) was subjected to successive silica gel CC eluted with increasing concentrations of CH_2Cl_2 in petroleum ether (100:0 to 10:90, v/v), followed by EtOAc in CH_2Cl_2 (100:0 to 10:90, v/v), and MeOH in EtOAc (100:0 to 80:20, v/v), as well as preparative TLC eluted with $CHCl_3$-EtOAc (90:10, three times) to give eudesmin (**5**, 32.6 mg; Batista et al., 2010), magnolin (**6**, 237.1 mg; Batista et al., 2010), and yangambin (**7**, 65.5 mg; Ahmed et al., 2002).

TLC investigations indicated a high concentration of alkaloids in the MeOH extract. Therefore, an aliquot of MeOH extract (40.0 g) was initially subjected to an acid-base extraction (Costa et al., 2006) to give alkaloid (0.67 g) and neutral (11.30 g) fractions. An aliquot of alkaloid fraction (0.50 g) was initially subjected to silica gel CC previously treated with a 10% $NaHCO_3$ solution (Costa et al., 2006), and eluted with increasing concentrations of CH_2Cl_2 in hexane (100:0 to 10:90, v/v), followed by EtOAc in CH_2Cl_2 (100:0 to 30:70, v/v), and MeOH in EtOAc (100:0 to 80:20, v/v), giving 218 fractions (15 mL each) that were evaluated and pooled according to TLC analysis to yield thirteen fractions. Fraction 8 (49.8 mg) was further purified on preparative TLC eluted with hexane-EtOAc (80:20, v/v, three times) affording eudesmin (**5**, 10.0 mg; Batista et al., 2010) and magnolin (**6**, 11.4 mg; Batista et al., 2010). Fraction 9 (64.3 mg) was also purified on preparative TLC eluted with hexane-EtOAc (60:40, v/v, four times) to yield eudesmin (**5**, 10.2 mg; Batista et al., 2010), magnolin (**6**, 17.0 mg; Batista et al., 2010), yangambin (**7**, 6.1 mg; Ahmed et al., 2002), nornuciferine (**8**, 5.3 mg; Guinaudeau et al., 1983; Chang et al., 2000), and lysicamine (**10**, 2.0 mg; Chang et al., 2000). Fraction 10 (14.4 mg) was subjected to preparative TLC eluted with CH_2Cl_2–MeOH (95:05, v/v, three times) resulting in lysicamine (**10**, 2.8 mg; Chang et al., 2000) and liriodenine (**11**, 2.4 mg; Chang et al., 2000; Costa et al., 2009a; Pinheiro et al., 2009). Fraction 11 (31.7 mg) was also subjected to preparative TLC eluted with CH_2Cl_2–MeOH (90:10, v/v, two times) giving asimilobine (**9**, 4.7 mg; Chang et al., 2000).

All isolated compounds (Fig. 1) were identified by a series of spectrometric methods, such as IR, UV, MS, and NMR (1D and 2D), as well as comparison with data reported in the literature. The complete and unequivocal NMR data for nornuciferine (**8**) were revised according to 1D and 2D NMR experiments, and are described in this work. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (1H, d, $J = 7.7$ Hz, H-11), 7.31 (1H, m, H-10), 7.23 (2H, m, H-9 and H-8), 6.65 (1H, s, H-3), 3.93 (1H, dd, $J = 13.7$ and 4.8 Hz, H-6a), 3.89 (3H, s, 2-OCH_3), 3.66 (3H, s, 1-OCH_3), 3.49 (1H, dd, $J = 12.0$ and 5.0 Hz, H-5pseudoeq), 3.12 (1H, m, H-4pseudoax), 3.06 (1H, dd, $J = 12.0$ and 2.6 Hz, H-5pseudoax), 2.97 (1H, dd, $J = 13.7$ and 4.8 Hz, H-7pseudoeq), 2.86 (1H, t, $J = 13.7$ Hz, H-7pseudoax), and 2.76 (1H, dd, $J = 15.8$ and 2.6 Hz, H-4pseudoeq); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.5 (C-2), 145.5 (C-1), 135.4 (C-7a), 132.0 (C-11a), 128.4 (C-11), 128.1 (C-3a), 127.9 (C-8), 127.5 (C-9), 127.2 (C-10), 127.0 (C-3b), 126.7 (C-1a), 111.7 (C-3), 60.2 (1-OCH_3), 55.9 (2-OCH_3), 53.4 (C-6a), 42.6 (C-5), 36.7 (C-7), and 28.3 (C-4).

4. Chemotaxonomic significance

The present work reports the isolation and identification of two sesquiterpenes (**1**–**2**), one diterpene (**3**), one steroid (**4**), three lignans (**5**–**7**), and four alkaloids (**8**–**11**): two aporphine (**8**–**9**) and two oxoaporphine (**10**–**11**) from the leaves of *A. pickelii* (Fig. 1). All isolated compounds are reported here for the first time from this plant. Caryophyllene oxide (**1**) and spathulenol (**2**) have been found to be components of essential oils from the leaves of several genera of Annonaceae, including *Annona* (Costa et al., 2009b, 2011; Pinheiro et al., 2009), *Duguetia* formerly known as *Pachypodanthium* (Boyom et al., 2003; Maia et al., 2006; Maas et al., 2011), *Guatteria* (Costa et al., 2008; Maia et al., 2005a), *Hexalobus* (Boyom et al., 2003), and *Xylopia* (Boyom et al., 2003; Maia et al., 2005b), and could be considered chemotaxonomic markers of these genera. Recently, they were described in the bark of *Annona salzmannii* (Da Cruz et al., 2011) and the stem of *Annona amazonica* (Pinheiro et al., 2009). Phytol (**3**) and β-sitosterol (**4**) are two compounds reported to occur in many plants (Dewick, 2009). Compounds **5**–**7** are three example of furofuranic lignans type found in few species of Annonaceae, particularly in the unrelated genera *Annona*, *Mitrephora* and *Porcelia* (Chen et al., 1996; Figueiredo et al., 1999; Chaves et al., 2000; Deepralard et al., 2007). In *Annona* they have been reported in the leaves and fresh unripe fruits of *Annona mucosa* (Chen et al., 1996; Figueiredo et al., 1999; Maas et al., 2011). Compound **5** was one of the first lignans described in Annonaceae from the leaves of *Annona sylvatica* (De Mesquita et al., 1988; Maas et al., 2011). Although lignans have been found in other closely related families, such as

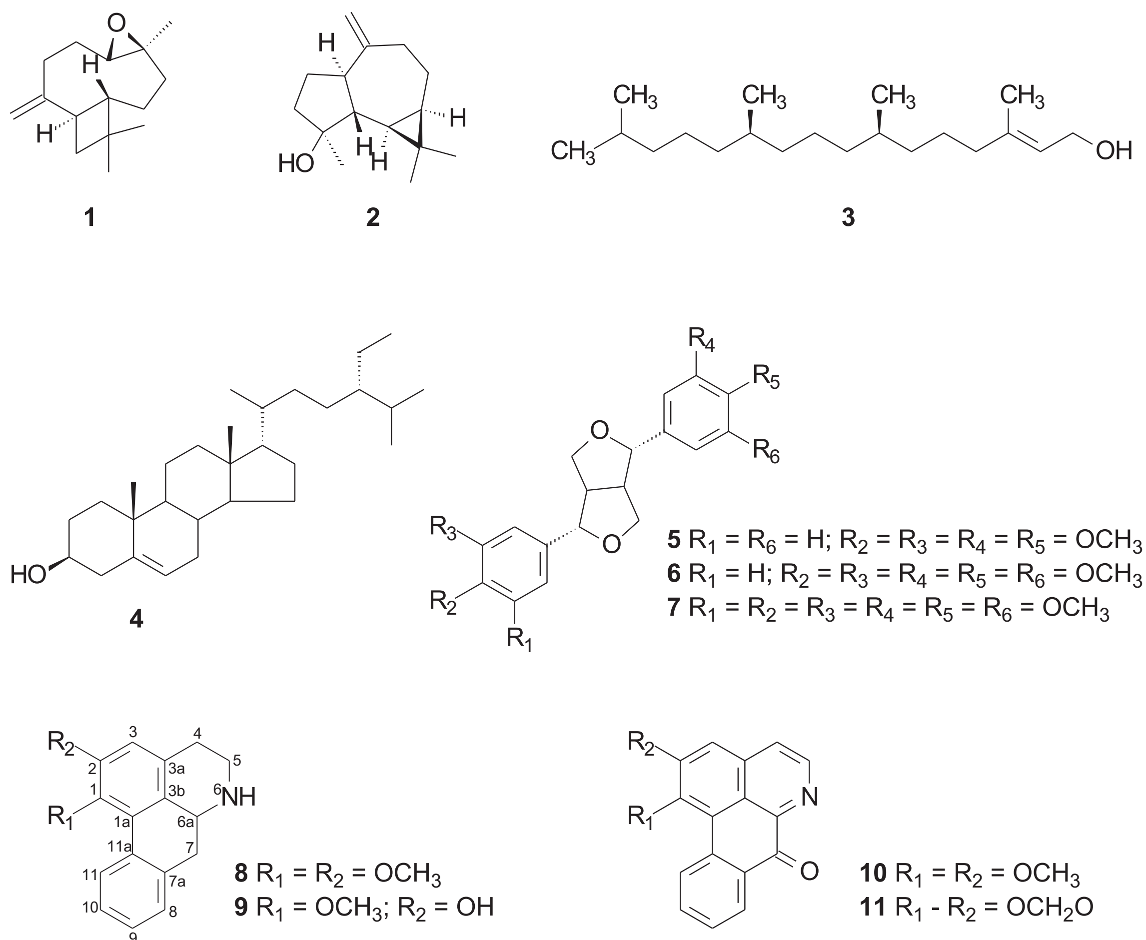

Fig. 1. Chemical constituents isolated from the leaves of *Annona pickelii*.

Magnoliaceae and Lauraceae, only a limited number have been reported from the Annonaceae (Leboeuf et al., 1982). Other examples of furofuranic lignans type in Annonaceae was found in the stems of *Annona montana* (Wu et al., 1995) and *Annona cherimola* (Chen et al., 1998), stem bark of *Annona emarginata* (Février et al., 1999), seeds of *Annona membranacea* (Saez et al., 1993), leaves of *A. pickelii* (De Mesquita et al., 1988), leaves and stems of *Mitrephora maingayi* (Deepralard et al., 2007), and branches of *Porcelia macrocarpa* (Chaves et al., 2000). Therefore, the presence of furofuranic lignans type in *Annona* suggests that these compounds could be used as potentially chemotaxonomic markers of this genus.

The aporphine [nornuciferine (**8**) and asimilobine (**9**)], and oxoaporphine [lysicamine (**10**), and liriodenine (**11**)] alkaloids are ubiquitous in the Annonaceae, and are found in all most genera of this family, mainly in the *Annona*, *Artabotrys*, *Desmos*, *Duguetia*, *Enantia*, *Fissistigma*, *Goniothalamus*, *Guatteria*, *Polyalthia* e *Xylopia* (Guinaudeau et al., 1975, 1979, 1983, 1988, 1994; Leboeuf et al., 1982; Costa et al., 2009c). They are considered as chemotaxonomic markers of the genera of Annonaceae, especially *Annona* (Da Cruz et al., 2011). In *Annona*, they have been reported in the leaves of *Annona sericea* (Campos et al., 2008), fresh fruits of *Annona glabra* (Chang et al., 2000), stems of *A. cherimola* (Chen et al., 1997), and bark of *A. salzmannii* (Da Cruz et al., 2011).

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