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Short communication

Alkaloids isolated from the leaves of atemoya (*Annona cherimola* × *Annona squamosa*)

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ABSTRACT

Atemoya is an interspecific annonaceous hybrid between *Annona cherimola* Mill. and *Annona squamosa* L. Its phytochemical investigation led to seven alkaloids, including two aporphine (anonaine and asimilobine), three oxoaporphine (lanuginosine, liriodenine and lysicamine) and two proaporphine (pronuciferine and stepharine). These alkaloids were identified by a series of spectrometric methods, mainly MS and NMR (1D and 2D), as well as by comparison with literature data. Our findings showed that this species is an important source of aporphine alkaloids and have high relationship with other *Annona* species.

Introduction

Annonaceae is a large family comprising about 135 genera and more than 2500 species distributed mainly in tropical and subtropical regions (Chatrou et al., 2004). Chemically, this family is characterized by the presence of isoquinoline alkaloids, mainly aporphines.

Regarding the species of Annonaceae, those from the genus *Annona* L. comprises approximately 175 species of trees and shrubs. In Brazil the genus *Annona* contains around 60 species, with the largest part occurring in forests and few representatives in open areas (Costa et al., 2011). Moreover, economically, this genus is the most important of the family Annonaceae due to its edible fruits and medicinal properties (Dutra et al., 2012).

Atemoya is a fruitful plant of the Annonaceae family, being a hybrid resulting from a cross between the "cherimoia" (*Annona cherimola* Mill.) and the "pinha" or "fruta-do-conde" (*Annona squamosa* L.). It was achieved at the beginning of the century in the Florida (USA), the crosses being repeated in other countries in order to obtain hybrids adapted to tropical climate, as *A. squamosa*, and subtropical as *A. cherimola* (Silva and Muniz, 2011).

The introduction of atemoya in the Northeast region of Brazil is recent, with a predominance of the cultivar Gefner from Israel, originally grown in the irrigation projects of the Vale do São Francisco. A recent study carried out by our research group quantified the levels of total phenols and flavonoids as well as evaluated the antioxidant and antimicrobial activities of extracts obtained from stems and leaves of atemoya (Rabêlo et al., 2014a).

Due to expansion of atemoya cultivation and its increasing consumption in the main Brazilian markets, it is necessary to investigate the chemical composition of this plant. In the present paper, we report results of the first phytochemical study of the atemoya collected in the Vale do São Francisco, and the isolation and chemical characterization of seven alkaloids by spectrometric methods.

Materials and methods

NMR experiments were acquired in $CDCl_3$, at 303 K on a Bruker AVANCE III 600 NMR spectrometer operating at 14.1 Tesla, observing ^1H and ^{13}C at 600 and 150 MHz, respectively. The spectrometer

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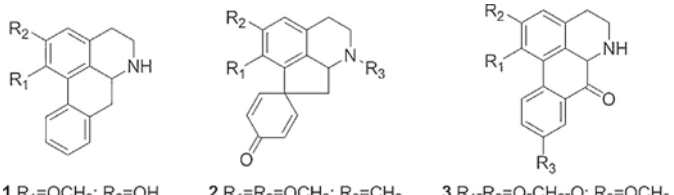
was equipped with a 5-mm multinuclear inverse detection probe with *z*-gradient. One-bond and long range $^{1}H–^{13}C$ correlation from HSQC and HMBC NMR experiments were optimized for average coupling constants $^{1}J_{(H,C)}$ and $^{LR}J_{(H,C)}$ of 140 and 8 Hz, respectively. All ^{1}H and ^{13}C NMR chemical shifts are given in ppm related to the TMS signal at 0.00 ppm as internal reference, and the coupling constants (*J*) in Hz. High-resolution ESI-MS data were taken in the positive ion mode, on a Bruker micrOTOF II – ESI-TOF Mass Spectrometer. Silica gel 60 (F_{254}) was used for analytical thin layer chromatography (TLC), while silica gel 60 (230–240 mesh) was used for column chromatography (CC). Spots on chromatograms were detected under exposure to UV light (254 and 365 nm). When necessary, Dragendorff's reagent was used to visualize the spots on the TLC plates.

Leaves of atemoya (*Annona cherimola* Mill. × *Annona squamosa* L.) were collected in Petrolina (Coordinates: 9°20′30″ S and 40°40′42″ W), state of Pernambuco, Brazil, in July of 2013. The species was identified by Prof. José Alves de Siqueira Filho, and a voucher specimen (16310) was deposited in the Herbário Vale do São Francisco of the Universidade Federal do Vale do São Francisco.

Dried and powdered leaves of atemoya (1163 g) were extracted with hexane (3 l, three times), followed by MeOH (3 l, three times), yielding of hexane (63 g) and MeOH (120 g) extracts, after each solvent removal under reduced pressure.

TLC analysis also indicated a high concentration of alkaloids in the MeOH extract, which was initially subjected to an acid–base extraction (Costa et al., 2006) to give alkaloid (1.5 g) and neutral (7.5 g) fractions. The alkaloidal fraction was subjected to silica gel CC previously treated with a 10% $NaHCO_3$ solution, eluted with increasing concentrations of hexane, CH_2Cl_2, EtOAc and MeOH, giving 297 fractions (40 ml each). These fractions were evaluated and pooled according to TLC analysis yielding eighteen groups. Group 5 (81.8 mg) was subjected to a preparative TLC eluted with CH_2Cl_2:MeOH (95:05, *v*/*v*, three times), giving asimilobine (**1**) (2.3 mg), a mixture of alkaloids asimilobine and pronuciferine (**2**) (6.5 mg), and a mixture of three oxoaporphine alkaloids lanuginosine (**3**), liriodenine (**4**) and lysicamine (**5**) (5.9 mg). Group 7 (66.9 mg) was subjected to a preparative TLC eluted with CH_2Cl_2:MeOH (95:05, *v*/*v*, three times), affording a mixture of alkaloids asimilobine and anonaine (**6**) (3.3 mg) and the proaporphine alkaloid stepharine (**7**) (2.5 mg).



All isolated compounds were identified by a series of spectrometric methods, mainly MS and NMR (1D and 2D) data, as well as comparison with those reported in the literature. Alkaloids in mixtures were established through totally independents datasets from the 1D and 2D NMR experiments including signal areas. Each dataset was consistent with only one structure. Moreover, it was observed the same results in several samples investigated.

Asimilobine (**1**): yellow amorphous solid, molecular formula $C_{17}H_{17}NO_2$. Positive ESI-MS *m*/*z*: 268.1354 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.27 (1H, *d*, *J* = 7.8 Hz, H-11), 7.22–7.33 (3H, *m*, H-8, 9, and 10), 6.72 (1H, *s*, H-3), 3.59 (3H, *s*, C1–OCH_3). The MS and ^{1}H NMR data are in agreement with the literature (Guo et al., 2011; Costa et al., 2015).

Pronuciferine (**2**): brown amorphous solid, molecular formula: $C_{19}H_{21}NO_3$. Positive ESI-MS *m*/*z*: 312.1284 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.41 (1H, *dd*, *J* = 8.1 and 2.8 Hz, H-12), 6.89 (1H, *dd*, *J* = 7.1 and 2.8 Hz, H-8), 7.00 (1H, *dd*, *J* = 8.1 and 2.8 Hz, H-9), 6.29 (1H, *dd*, *J* = 8.10 and 2.00 Hz, H-11), 6.64 (1H, *s*, H-3), 3.60 (3H, *s*, C1–OCH_3), 3.80 (3H, *s*, C2–OCH_3), 3.05 (3H, *s*, N–CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 184.42 (C-10), 153.34 (C-8), 128.49 (C-9), 127.10 (C-11), 149.80 (C-12), 61.15 (1-OMe), 56.48 (2-OMe), 44.83 (N-Me). The MS, ^{1}H NMR and ^{13}C NMR data are in agreement with the literature (Thuy et al., 2005).

Lanuginosine (**3**): dark brown solid, molecular formula: $C_{18}H_{11}NO_4$. Positive ESI-MS *m*/*z*: 306.0762 $[M+H]^+$. The MS data are in agreement with the literature (Wijeratne et al., 1996).

Liriodenine (**4**): yellow amorphous solid, molecular formula: $C_{17}H_9NO_3$. Positive ESI-MS *m*/*z*: 276.0659 $[M+H]^+$. The MS data are in agreement with the literature (Guo et al., 2011; Costa et al., 2011).

Lysicamine (**5**): yellow amorphous solid, molecular formula: $C_{18}H_{13}NO_3$. Positive ESI-MS *m*/*z*: 292.0971 $[M+H]^+$. The MS data are in agreement with the literature (Harrigan et al., 1994).

Anonaine (**6**): yellow powder, molecular formula: $C_{17}H_{15}NO_2$. Positive ESI-MS *m*/*z*: 266 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.15 (1H, *d*, *J* = 7.74 Hz, H-11), 7.22–7.32 (3H, *m*, H-8, 9, 10), 6.58 (1H, *s*, H-3), 6.09 and 5.94 (each 1H, *s*, –OCH_2O–). The MS and ^{1}H NMR data are in agreement with the literature (Guo et al., 2011; Costa et al., 2015).

Stepharine (**7**): amorphous brown solid, molecular formula: $C_{18}H_{19}NO_3$. Positive ESI-MS *m*/*z*: 298.1438 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.39 (1H, *dd*, *J* = 8.4 and 2.0 Hz, H-12), 6.87 (1H, *dd*, *J* = 7.4 and 2.0 Hz, H-8), 6.98 (1H, *dd*, *J* = 7.4 and 2.00 Hz, H-9), 6.28 (1H, *dd*, *J* = 8.4 and 2.0 Hz, H-11), 6.64 (1H, *s*, H-3), 3.60 (3H, *s*, C1–OCH_3), 3.80 (3H, *s*, C2–OCH_3). The MS and ^{1}H NMR data are in agreement with the literature (Thuy et al., 2005; Costa et al., 2015).

Results and discussion

The phytochemical investigation from the leaves of the hybrid atemoya, an edible fruit of the Annonaceae family, resulted in seven alkaloids, including two aporphine (anonaine and asimilobine), three oxoaporphine (lanuginosine, liriodenine and lysicamine) and two proaporphine (pronuciferine and stepharine). All of them are being described for the first time in the hybrid atemoya.

This family is considered the centre of distribution of isoquinoline alkaloids. Recent chapter published by our research group presented an overview of the chemistry and pharmacology of the alkaloids found in species of the Annonaceae family. Within the Annonaceae family, the genera *Annona*, *Duguetia*, and *Guatteria* have led to many important publications. The alkaloids of the aporphine type represent the predominant group in this family. Many of the isolated alkaloids exhibit unique structures. The chapter could be considered as a contribution for the scientific community, mainly to enable the search for alkaloids in species belonging to the Annonaceae family (Lúcio et al., 2015). Another work reviewed articles published in the literature regarding alkaloids isolated from plants of the genus *Annona*. This review covers a period from 1930 to 2013 and shows the identification of 147 alkaloids in *Annona* species (Rabêlo et al., 2014b).

The aporphine asimilobine and anonaine, and the oxoaporphine liriodenine have been described in several species of *Annona*. Recent studies describe the isolation of asimilobine and liriodenine in some species of *Annona*, such as *Annona foetida* Mart., *Annona pickelii* (Diels) H. Rainer, *Annona salzmannii* A. DC., and *Annona sericea* Dunal, all native to Brazil (Campos et al., 2008; Costa et al., 2011; Cruz et al., 2011; Dutra et al., 2012). These alkaloids were found in leaves and barks of *A. crassiflora* from the Guianas (Hocquemiller et al., 1982). Anonaine, asimilobine and liriodenine have been described in several species of *Annona*, and could be

considered as chemotaxonomic markers of this genus (Cruz et al., 2011).

Other studies described the isolation of the alkaloid pronuciferine in *Annona cherimola* (Chen et al., 1999); lanuginosine in *A. cherimola*, *A. rugulosa* and *A. squamosa;* lysicamine in *A. acuminata*, *A. cherimola*, *A. glabra*, *A. hayesii*, *A. pickelii*, *A. purpurea* and *A. sericea*; and stepharine in *Annona cacans*, *A. glabra*, *A. hayesii* and *A. spinescens* (Rabêlo et al., 2014b). All these compounds are being described for the first time in the hybrid atemoya collected in the Vale do São Francisco.

Authors' contributions

SVR, XPN and MFCS were responsible for the collection, preparation of the extracts and phytochemical studies. EVC, AB and LMD conducted the experiments of nuclear magnetic resonance. JCT, GGO and NPL conducted the experiments of mass spectrometry. EVC and JRGSA analyzed and interpreted the data, and drafted the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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