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Annonaceous acetogenins reported for the first time in the leaves and fruit's pulp of Annona Atemoya

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Abstract:

Annona genus is a member of Annonaceae family that encompassing about 175 species and most of them are native of Brazil and tropical America. Due to its edible fruits and medicinal proprieties, this genus is the most important among of Annonaceae family. Annona atemoya (A. atemoya) belongs to the family of Annonaceae known as custard apple and a species of the genus Annona. This plant is commonly found in tropical and subtropical continents such as Asia, Africa, southern and northern America and Australia. Since 1907, A. atemoya has been known as hybridization of two Annona species: the sugar apple (Annona squamosa) and Cherimoya (Annona cherimola. Preliminary results of TLC and NMR experiments indicated the presence of annonaceous acetogenins for the first time in the leaves and pulp as pink bands after reacting with Kedde reagent. For the leaves, ten compounds were identified, two of them were isolated and the other confirmed via NMR and MS analysis. With regards to the fruit's pulp, the concentration of acetogenins was very low and compared to the leaves. Future studies will focus on testing either isolated compounds or crude extracts using various cancer cell lines.

Keywords: Annonaceae, Annona atemoya, acetogenins, leaves, fruit

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Introduction





Material and methods

✤ Plant material collection and preparation

- The fully matured fresh leaves and fruits of *A. atemoya* were collected in July 2020 from a local farm in Queensland.
- Both leaves and fruits were washed with running tap water to cleanness and then kept on the bench for air drying at room temperature and well-ventilated area for one week.
- Then, the leaves and fruits were crushed into small pieces and powdered via an electric blender (Multifunctional crusher).
- The final volume for the leaves and fruits powder were 222.4 g and 380 g, respectively.



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Preparation of plant extracts

- The powders of leaves and fruits were macerated firstly with hexane, followed by extraction them in ethyl acetate and finally with EtOH.
- All extracts were shaken by using an electronic stirrer and kept for 72 hours and then filtrated using vacuum filtration and Buchner funnel.
- The final crude extracts for leaves were 12.3 g, 3.7 g and 24.7 g.
- For the fruit's pulp, the final crude extracts were 2.06 g, 0.7 g and 25.52 g.



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* Isolated compounds: Squamocin G



* Isolated compounds: Squamocin C



 ^1H NMR and ^{13}C [CDCl_3, 400 MHz], chemical shift for squamocin C





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- In the MS, a molecular mass was determined as 530 by ESI-MS ($[M+H]^+ m/z = 531$) leading to the molecular formula $C_{35}H_{62}O_3$ suggested Epoxymurin-A.
- The MSMS was also confirmed the presence of product ions spectrum of [M + Li]⁺ (*m*/*z* = 537) and intense fragments at *m*/*z* 313, 301, 285 and 271.
- Another compound was also identified only in the ethyl acetate extract was Montanacin A and showed a doublet peak at 7.20 ppm, and at $[M + Li]^+$ (m/z = 647) leading to the molecular formula $C_{37}H_{68}O_8$.
- The MSMS analysis revealed fragments at *m*/*z* 535, 463, 337, 277 and 205 suggested the localization of mono-THF ring at C-20 and C-23.

Conclusions

- To our knowledge, this is the first quantitative determination of acetogenins in the leaves and fruits of *A. atemoya*.
- All acetogenins (1-11) were identified putatively for the first time in the leaves of *A. atemoya*.
- Four acetogenins (1-4) were reported in both extracts in common.
- Among of the reported acetogenins three were epoxy type with mono-epoxy compound (1) and bis-epoxy compounds (2 and 3).
- Finally, three compounds (4, 5 and 8) were reported for having a mono-THF, four compounds with adjacent bis-THF compounds (6, 7, 9 and 11), and one with non-adjacent bis-THF compound (10).

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