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Preliminary studies on phytochemical constituents of the leaves and fruits of *Annona atemoya*

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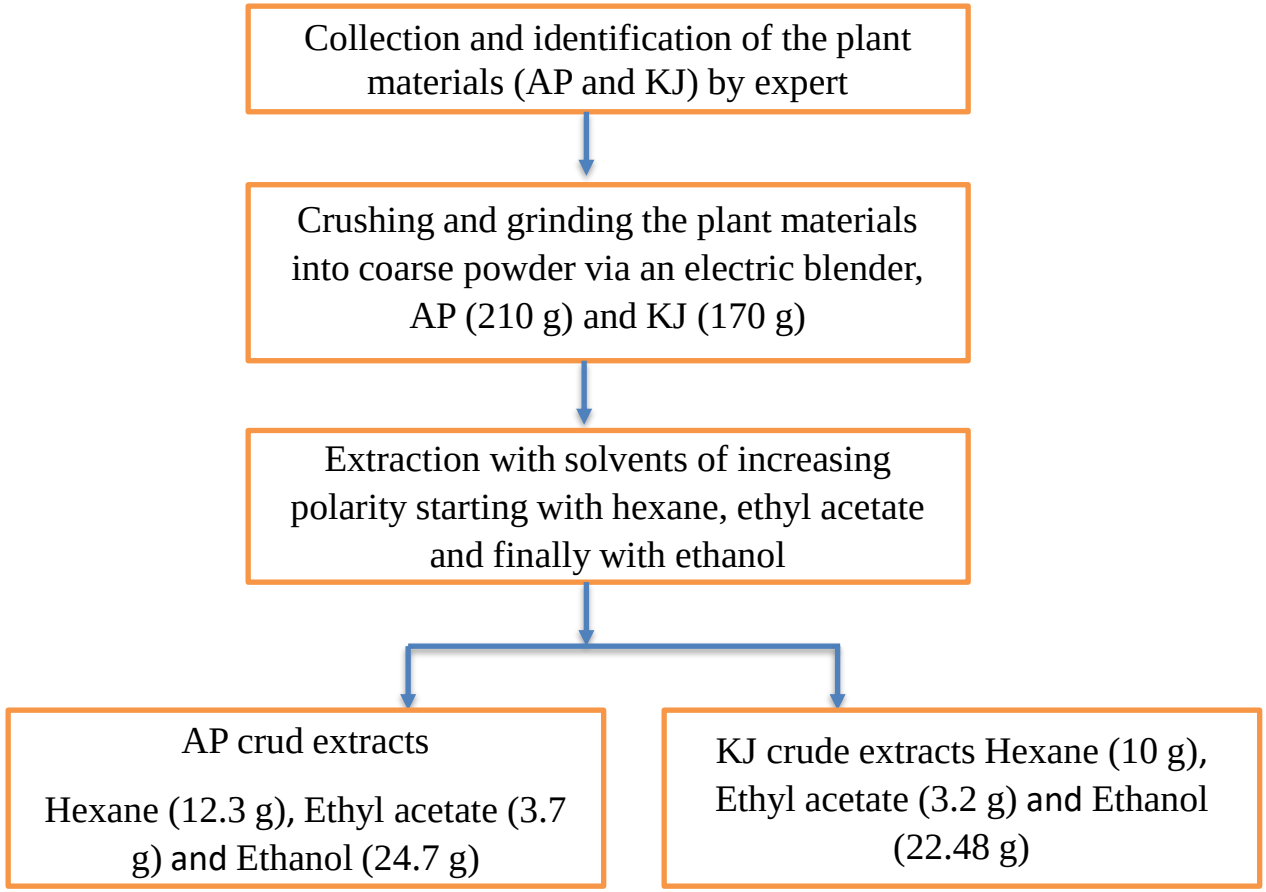
Graphical Abstrat

Schematic presentation of extraction performed on *A.atemoya* leaves (AP and KJ).



Abstract

Annona atemoya is a commercially important fruiting plant belonging to the Annonaceae family. It is widely cultivated in tropical and subtropical continents. It is also known as the custard apple which is a hybrid between two Annonaceae species: (*Annona cherimola*) and (*Annona squamosa*). This study aimed to investigate the phytochemical constituents and pharmacological activity of various parts of *A. atemoya* including leaves, fruits and seeds. The leaves and fruits of *A. atemoya* were collected in July 2020 from a local farm in Queensland, air dried at room temperature (3 days – 1 week) and then ground to a powder. The leaves and fruits were separately extracted with hexane, ethyl acetate and finally ethanol for three days each. Preliminary results of TLC and NMR experiments indicated the presence of annonaceous acetogenins for the first time in all extracts as pink bands after reacting with Kedde reagent. For the leaves, ethanolic extraction yielded the most polar acetogenins and the ethyl acetate extract was the richest in an abundance of acetogenins containing approximately three separated bands in comparison to the ethanolic and hexane extracts. With regards to the hexane extraction, the concentration of acetogenins was very low and demonstrated the lowest polar acetogenins. All extracts were further purified by chromatography. Future studies will focus on the isolation and characterization of these components and testing of biological activities on various cancer cell lines.

Keywords: *Annona atemoya*, annonaceous acetogenins, isolation, biological activity.

Introduction

- *Annona atemoya* is a commercially important fruiting plant belonging to the Annonaceae family.
- It is widely cultivated in tropical and subtropical continents.
- It is a hybrid between two Annon species: Cherimoya (*Annona cherimola*) and the sugar apple (*Annona squamosa*).
- Hybridization was conducted by P. J. Webster in Florida (USA) between 1907 and 1908.
- The traditional uses of *A. atemoya* are lacking, with some limited anecdotal evidence that the leaves of *A. atemoya* are purchased from growers for making teas.

Introduction

Country of origin	Selection and cultivar
USA Florida/California	Bradley, Page, Keller, Priestly, Stremer and Caves
Australia	Island Gem, Nielsen, Hillary White, Maroochy Gold, Pink's Mammoth and African Pride
Egypt	Finny and Cherimata
Thailand	Golden Flesh and Pet Pakchong
Israel	Jennifer, Kabri, Malalai, African Pride and Gefner
South Africa	African Pride
Brazil	African Pride, Pink's Mammoth, Thompson and Gefner

Introduction

- Several phytochemical constituents have been isolated from different parts of the *A. atemoya* plant and assessed for their biological potential in both *vivo* and *vitro* studies.
- The leaves, fruits and seeds of *A. atemoya* are the most widely studied for their chemical and pharmacological properties.
- However, only limited studies have investigated the phytochemistry and bioactivities of *A. atemoya.*
- Therefore, the aims of this study are investigating the phytochemical constituents of the leaves, fruits and seeds of *A. atemoya* and evaluating the activity of isolated compounds using cancer cell lines.

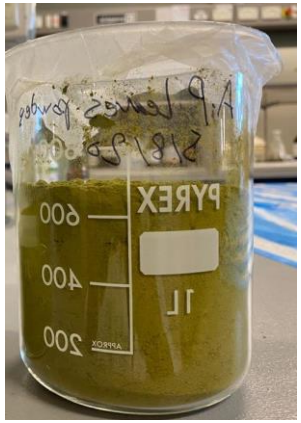
Material and methods

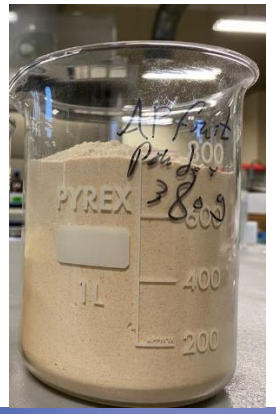
- **Plant material collection and preparation**
- The fully matured fresh leaves of both *A. atemoya* cultivars (AP) and (KJ) were collected in July 2020 from a local farm in Queensland.
- The leaves were washed with running tap water to cleanness and then kept on the bench for air drying at room temperature and well-ventilated area for one week.
- Then, the leaves were crushed into small pieces and powdered via an electric blender (Multifunctional crusher).
- The final volume of the (AP) and (KJ) leaves powder is 222.4 g and 180.9 g, respectively.

Material and methods

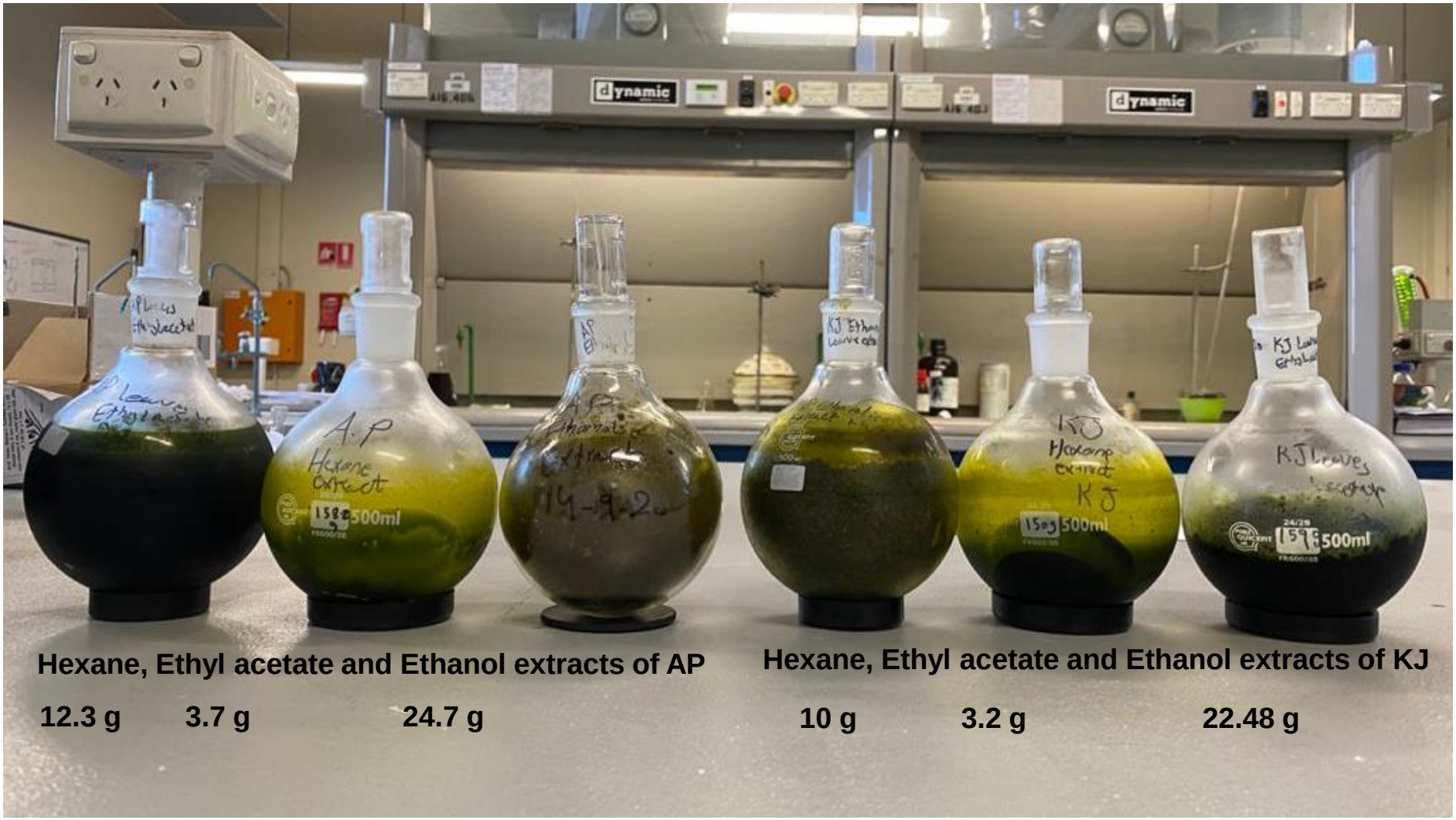
- **Preparation of plant extracts**
- The dried and powdered leaves of both (AP) 210 g and (KJ) 170 g were macerated firstly with hexane (2.5 L) and (2 L) respectively, followed by extraction them in ethyl acetate, and finally with EtOH.
- All extracts were shaken by using an electronic stirrer and kept for 72 hours and then filtrated using vacuum filtration and Buchner funnel.
- The solvents were evaporated by using a rotary evaporator yielding hexane 10 g for (KJ) and 12.3 g for (AP), ethyl acetate 3.2 g for (KJ) and 3.7 g for (AP) and EtOH 22.48 g for (KJ) and 24.7 g for (AP).

Material and methods





Results and discussion



Hexane, Ethyl acetate and Ethanol extracts of AP

12.3 g 3.7 g 24.7 g

Hexane, Ethyl acetate and Ethanol extracts of KJ

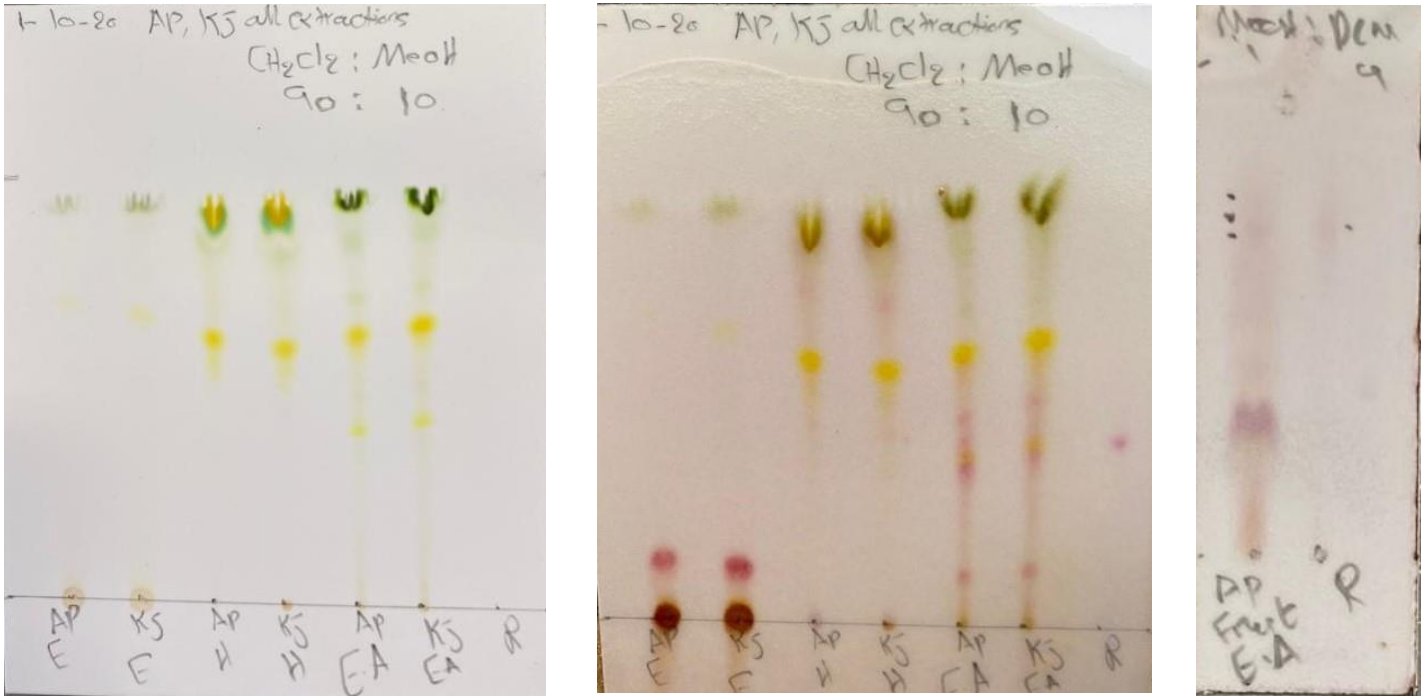
10 g 3.2 g 22.48 g

Results and discussion

- **Thin Layer Chromatography (TLC)**
- All six extracts of both AP and KJ leaves indicated the presence of acetogenins as pink bands after reacting with Kedde reagents.
- Both cultivars indicated some similarities in their phytochemical components as they displayed similar bands on the TLC plate.
- Both cultivars ethanolic extractions exhibited the same bands of the most polar acetogenins and have *Rf* at (0.25).
- Ethyl acetate extractions of both cultivars also illustrated the same bands of the high polar acetogenins at (0.25), however, the spots were less concentrated compared to the ethanolic extractions.
- The ethyl acetate extractions of AP and KJ were the richest in an abundance of acetogenins and contain approximately three separated bands in comparison to the ethanolic and hexanolic extracts.

Results and discussion

- **Thin Layer Chromatography (TLC) for the leaves**



TLC profile of total *A.atemoya* cultivars (AP and KJ), from left to right AP and KJ ethanolic extractions, then, AP and KJ hexanolic extractions, AP and KJ ethyl acetate extractions, AP fruit ethyl acetate extract and (R) (annonacin) after spraying with Kedde reagents.

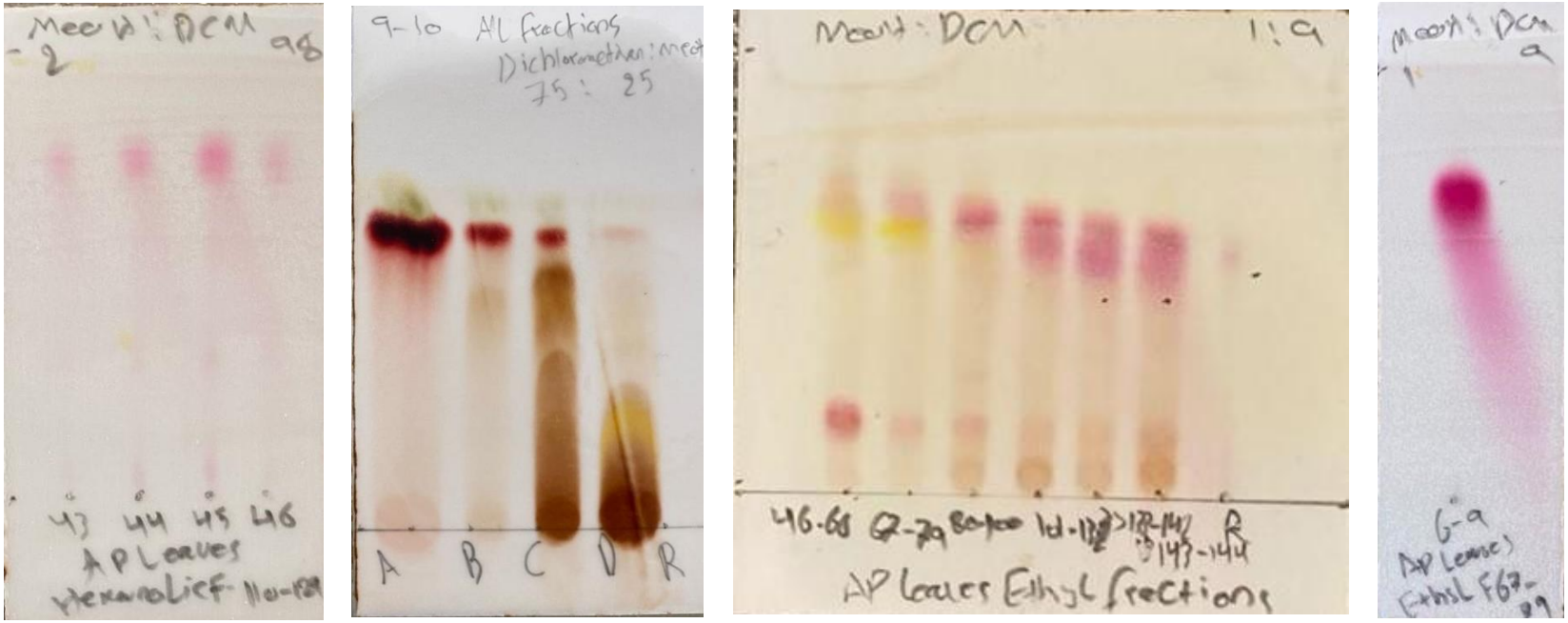
Results and discussion

- **Column Chromatography (CC) and flash chromatography**

Extraction	Fractions indicated acetogenins with major contamination	Eluting system	Subfractions indicated acetogenins	Eluting system	Subfractions indicated acetogenins
Hexane extract	From 110 to 129 (145.2 mg)	(DCM: MeOH) (100: 0 v/v) to (DCM: MeOH) (0: 100 v/v)	From 44 to 46 (39.8 mg)	(Hexane: EtOAc) (100: 0 v/v) to (Hexane: EtOAc) (0: 100 v/v)	Subfractions 43 (1.5 mg), 44 (3.5 mg), 45 (4.3 mg) and 46 (0.8 mg)
	Fractions 130 and 131 (33.3 mg)	(DCM: EtOH) (100: 0 v/v) to (DCM: EtOH) (0: 100 v/v)	From 18 to 21 (3 mg)	Due to the small amount, they will be submitted for MS analysis	
	From 138 to 143 (24 mg)	(DCM: EtOH) (100: 0 v/v) to (DCM: EtOH) (0: 100 v/v)	From 35 to 38 (3.4 mg)		
Ethanol extract	Fraction B (80 mg)	(DCM: MeOH) (100: 0 v/v) to (DCM: MeOH) (0: 100 v/v)	From 37 to 43 (18 mg)	(DCM: EtOAc) (100: 0 v/v) to (DCM: EtOAc) (0: 100 v/v)	From 16 to 28 (1.4 mg) and from 30 to 36 (1.8 mg)
Ethyl acetate extract	From 46 to 66 (121.4 mg)	(DCM: MeOH) (100: 0 v/v) to (DCM: MeOH) (0: 100 v/v)	From 37 to 44 (12.2 mg), From 45 to 55 (6 mg)	(DCM: EtOAc) (100: 0 v/v) to (DCM: EtOAc) (0: 100 v/v)	Subfractions 71 and 72 (9 mg)
	From 67 to 79 (66.7 mg)	(DCM: EtOAc) (100: 0 v/v) to (DCM: EtOAc) (0: 100 v/v)	Subfraction 25 (44.3 mg)	(100% EtOAc)	From 6 to 9 (11.2 mg)

Results and discussion

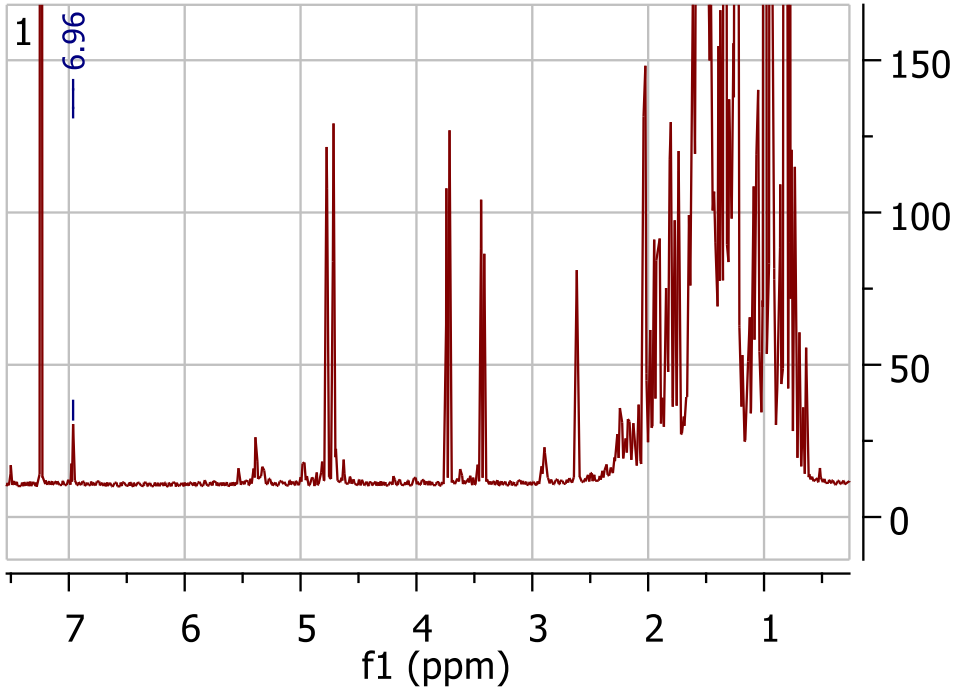
- **Column Chromatography (CC) and flash chromatography**

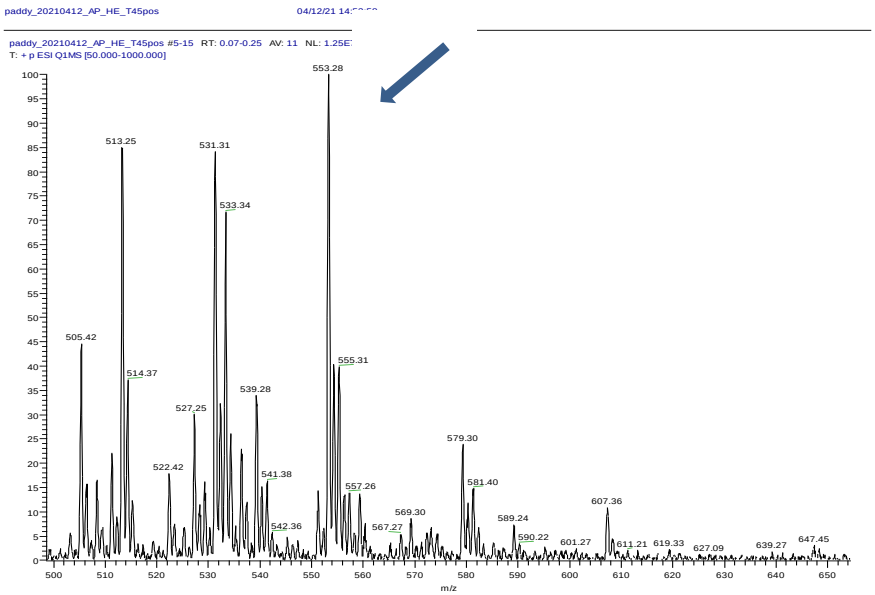


TLC plats for AP leaves hexanolic fractions (110 to 129) subfractions (43, 44, 45 and 46), AP leaves ethanolic fractions, AP leaves ethyl acetate fractions (46 to 144) and AP leaves ethyl acetate fractions (67 to 79) subfractions (6 to 9)

Results and discussion

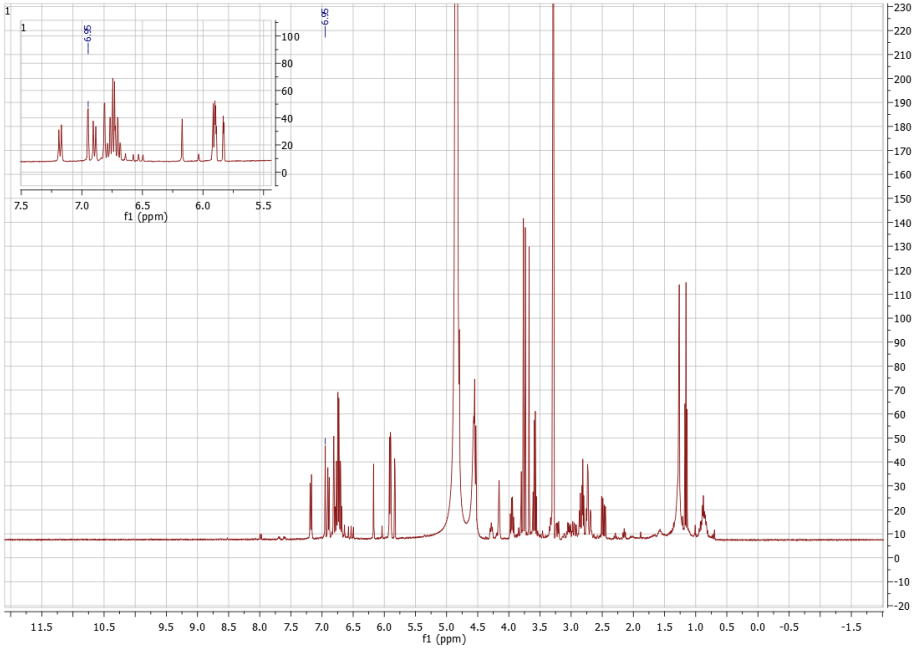
- **Nuclear Magnetic Resonance (NMR) and Mass Spectrometry:** Hexanolic subfractions 45, Epomuricenins-A and -B

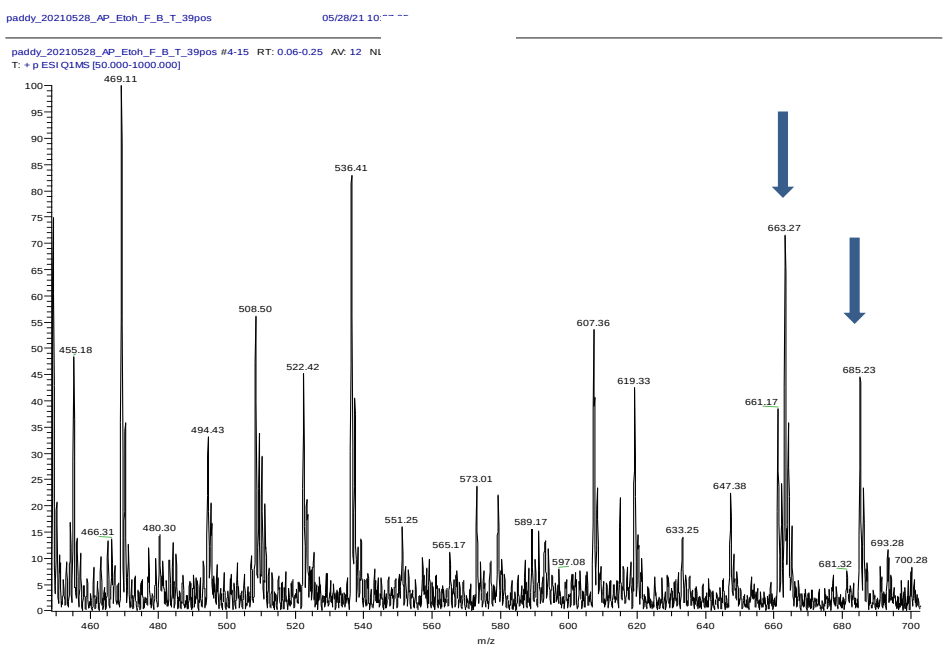




Results and discussion

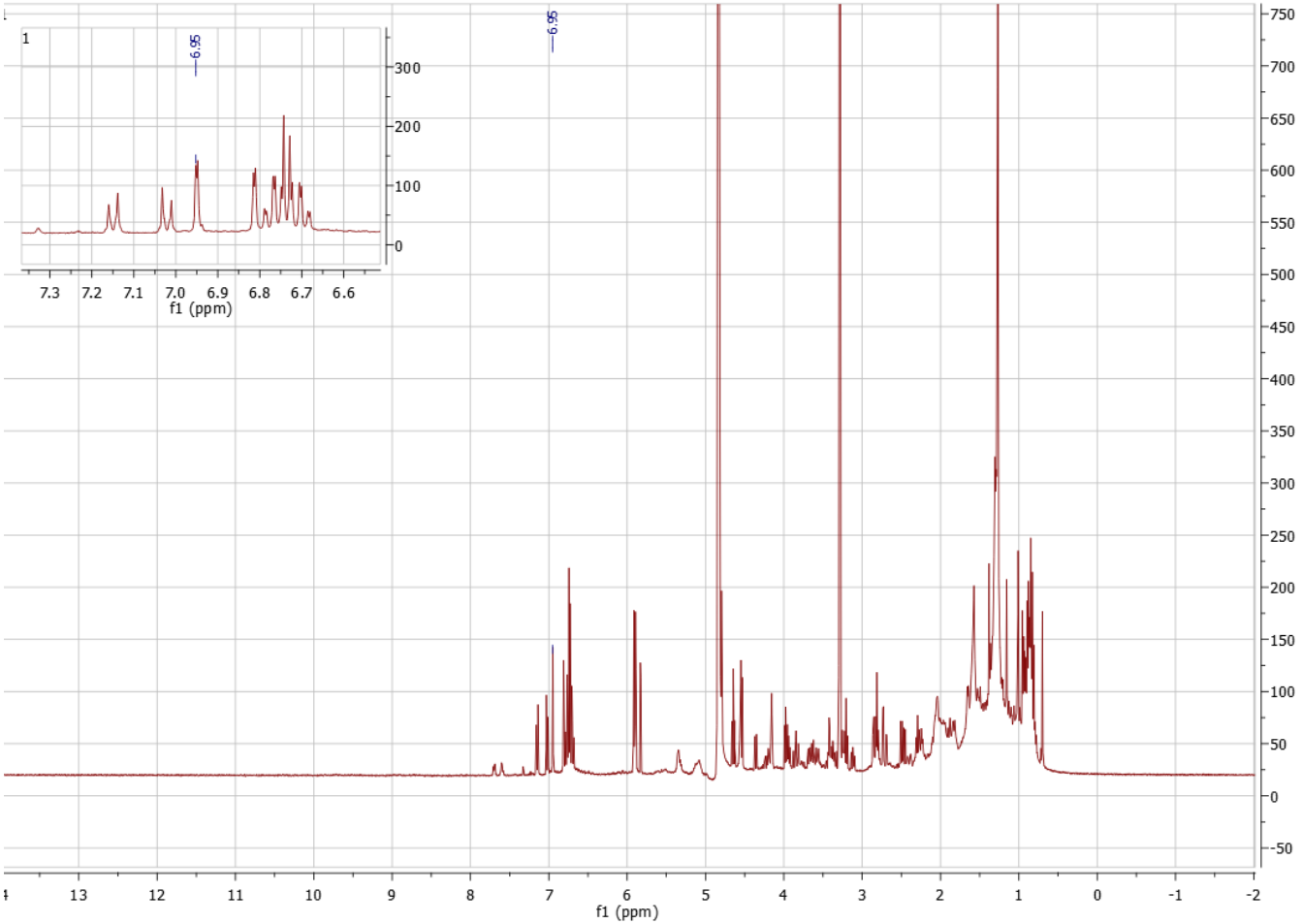
- **Nuclear Magnetic Resonance (NMR) and Mass Spectrometry:** Ethanolic fractions B, Squadiolin A and B, 15-Acetyl-guanacone and 24-Acetyl-guanacone





Results and discussion

- **Nuclear Magnetic Resonance (NMR):** Ethyl acetate subfractions 46-66



Conclusions

- To our knowledge, this is the first time to report the presence of acetogenins in both leaves and fruits of *A. atemoya*.

- Further studies are recommended in order to isolate and investigate the pure components and identify their activities on cancer cell lines.

Acknowledgments

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