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Original Article

Caribbean Soursop (*Annona Muricata*) Varieties II: Annonaceous Acetogenin Properties

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Abstract

The use of Annona muricata (graviola) as a tea decoction for the treatment of cancer and as broad spectrum antimicrobial, as an anti-internal parasite treatment and for lowering blood pressure has continued to increase. This study evaluated extracts of the leaf, fruit, seed, bark and roots of two types of fruit, which have high fibre, or fibreless fruit and are cultivated locally. The alcohol extracts of the different plant parts were analyzed by LC-MS to determine the phyto-chemical and cytotoxic (anti-tumor) properties and to classify them into the group of active annonaceous acetogenins. The samples were subjected to 3 [MS –ve, +ve mode, and UV] chromatograms. The results indicated the presence of confirmed acetogenins — Annomutacin or cis-Annomontacin [10.26 with MW about 625], and the cyclic peptide called annomuricatin [MW 11.05] — in the seeds. The leaf contained the similar compounds as the seed with a few other things at MW 300. The UV spectrum identified two phenolic chromphores, and several flavonoid type compounds. Basically, there were more flavonoid type compounds in the leaf extracts and fewer, if any, acetogenins. Based on the molecular weight, there are new compounds, which are found in the root and bark samples. Acetogenins are of great interest due to their cytotoxic properties (anti-cancer) and their toxicity to insects.

Keywords: Graviola, Acetogenins, Annomutacin, cis-Annomontacin, LC-MS.

Introduction

Soursop (*Annona muricata*) also called graviola or guanabana is an evergreen native tropical plant that produces a long green prickly fruit with white sub acidic pulp and is used in fresh juices, sherbets or as a desert fruit[1]. It is an accepted ethno-medicinal remedy for a range of ailments due to its anti-microbial; [2] antifungal, anti–depressant and lowering of blood pressure properties[3] and 'selective cytoxicity'. [4, 5] The fruit is also consumed with the cultural and folkloric understanding that it has anti-cancer properties. *A.muricata* contains both isoquinolic alkaloids and acetogenins, which are a large unique and structurally homogenous class of polypetides specific to Annonaceae.[6]

Annonaceous acetogenins are present in the leaf and stem, bark, and fruit seeds [6, 7] and include muricins A–G [1–7][8,3] muricatetrocin A[8], muricatetrocin B[3], longifolicin[9], corossolin and corossolone.[10] These acetogenins have showed significant selective *in-vitro* cytotoxicities toward the human hepatoma cell lines Hep G2 and 2,2,15 9.

Several compounds were isolated from Annona fruits *viz*: cis-annonacin, arianacin and javorcis some of which were selectively cytotoxic to colon adeno carcinoma, pancreatic carcinoma cells (PACA-2), lung carcinoma cells (A-549), all with potencies equal to or exceeding those of Adriamycin. Two acetogenins were isolated from the leaves *viz*: muricoreacin and murihexocin[12]. In the pulp extracts, in addition to the previously discovered eopmuricenins –A and –B, two new mono-epoxy saturated C-35 representatives[13]. More recently, the isolation and confirmation of 2 acetogenins – *Annomutacin* or *cis-Annomontacin* and *annomuricatin* were made in the leaf and seed respectively in Annona.[14, 15] However it was shown that there were more flavonoid type compounds in the leaf extracts and fewer if any acetogenins (Table 1). This new information dispels the ethno-medicinal belief that the soursop leaf teas can be used in cancer prevention or treatment. The aim of this study is to characterize the acetogenins profile of two varieties of soursop that are cultivated in the Caribbean.

Materials and Methods

The plant parts for this study were harvested from a 2 acre orchard established at the Waterloo Research Campus, University of Trinidad and Tobago. The trees were over 8 years old and were bearing. They were planted at a spacing

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Table 1. Annonaceous acetogenins present in two varieties of Caribbean Soursop [15]

Plant Parts	**Annonaceous acetogenins**	
	Fibre	**Fibreless**
Seeds	Annomontacin [10.26MW at 625]	Annomontacin [10.26MW at 625]
	Annomuricatum	Annomuricatum
	Annonamutacin	Annonamutacin
	—	New compound [furan ring MW 596 , and MW=566 (M+H or M+Na or M-H+Formate]
	New compound [MW596, tetrahydrofuran acetogenim at MW596]	New compound [MW596, tetrahydrofuran acetogenim at MW596]
Leaves	• More flavonoid compounds and fever if any acetogenins. • Poor sources of cyto-toxics.	

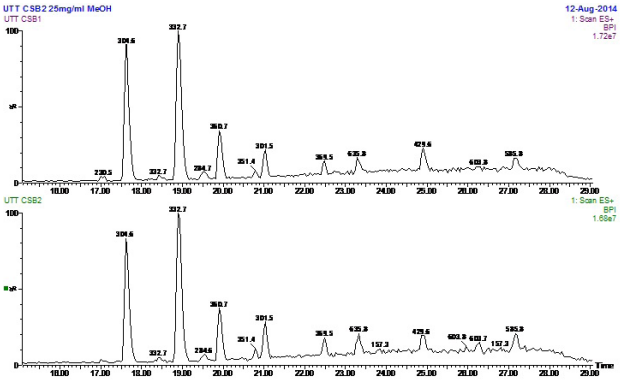

Figure 1. Chromatograms and associated spectra for ethanolic bark extracts of non fibre [CSB 1 and 2] variety [B] of *A. muricata*.

of 6m x 6m. The crop was managed without the input of fertilizer or other pesticides. The fully mature fruits of the two varieties of Annona used in this study are *A. muricata* var. *Cuba*, and *A. muricata* var. *Grenada* each with distinct fiber content. Var. *Grenada* is characterized with a high fibre [FS] , whilst var. *Cuba* is distinctly fibreless [CS]. The ripe fruits were harvested and de-pulped [CSP and FSP] to separate the seeds and outer skin. From the trees of both varieties, strips of plant bark [CSR and FSR] and roots [CSR and FSR] were removed and air dried for 72 hours in full sunlight and refrigerated at 0°C. The samples (200 g) of pulp [P], bark [B], and roots [R] were pulverized separately and then soaked in 200 mL of 95% ethanol for 2 hours at room temperature. The ethanolic extracts were assessed at 15 mg/mL or a 25 mg/mL concentration in duplicate of the same batch using LC-MS. [16] The analysis were conducted at the Kew Gardens and Natural Resources Institute in England, where the phyto-chemical and cytotoxic [anti-tumor] profiles were classified into the group of active *annonaceous acetogenins*. Each sample is comprised of chromatograms: an MS in –ve mode, MS in +ve mode and then the UV chromatogram. In general,

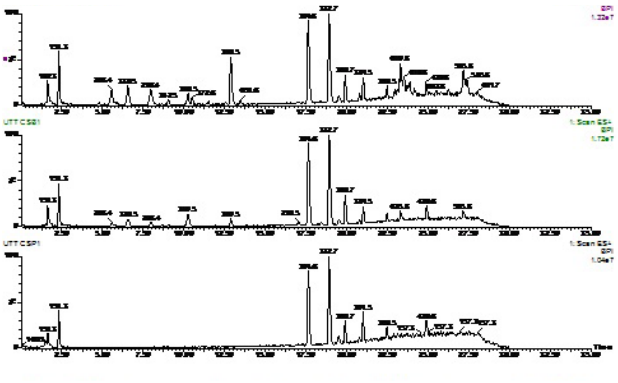

Figure 2. Chromatograms and associated spectra for ethanolic root extracts of high fibre [FSR1], and bark extracts of non fibre variety [CSB1], and pulp [CSP1] of *A. muricata*.

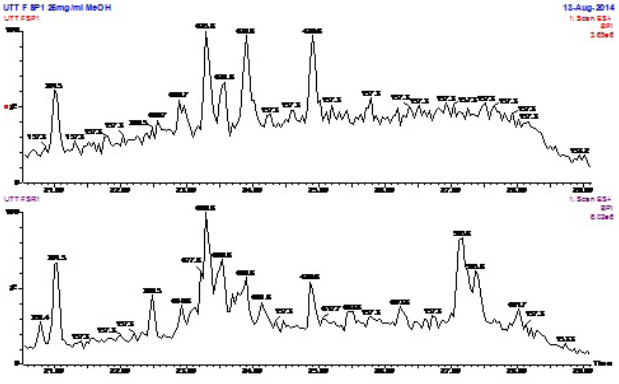

Figure 3. Chromatograms and associated spectra for ethanolic extracts of high fibre pulp [FSP1], bark [FSB1], and root [FSR1] and bark extracts of non fibre variety [CSB1], and pulp [CSP1] of *A. muricata*.

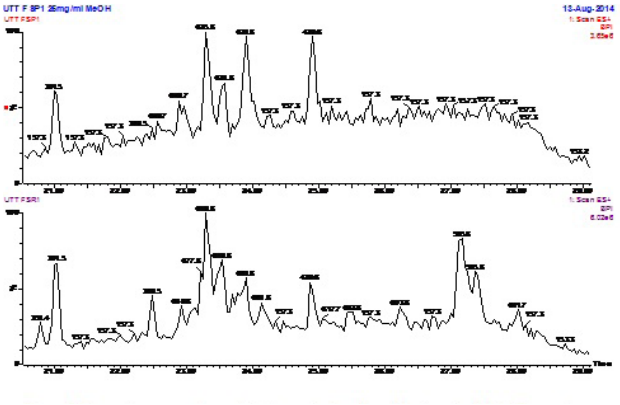

Figure 4. Chromatograms and associated spectra for ethanolic extracts of high fibre pulp [FSP1], root [FSR1] of *A. muricata*.

the MS in -ve will give an M-H ion or an M-H+formate. Sometimes they give dimers. In general the +ve mode gives M+H or M+Na and also sometimes dimers.[17,18]

Results

The chromatograms and associated spectra of ethanolic extracts of the two varieties of annona used in this study referred to as high fibre [FS] and fibre free [CS] for the varying plant parts root [R], bark [B] or pulp[P] are presented in Figures 1 to 7.

In this study, the actogenin compounds are those that

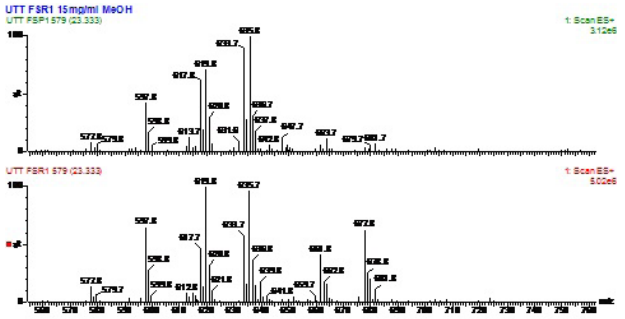

Figure 5. Chromatograms and associated spectra for ethanolic extracts of high fibre pulp [FSP1], root [FSR1] of *A. muricata*.

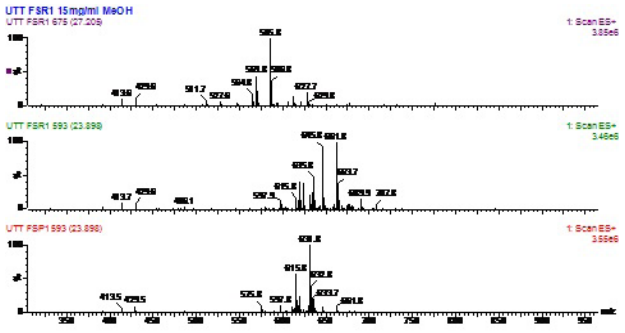

Figure 6. Chromatograms and associated spectra for ethanolic extracts of high fibre root [FSR1], pulp [FSP1] of *A. muricata*.

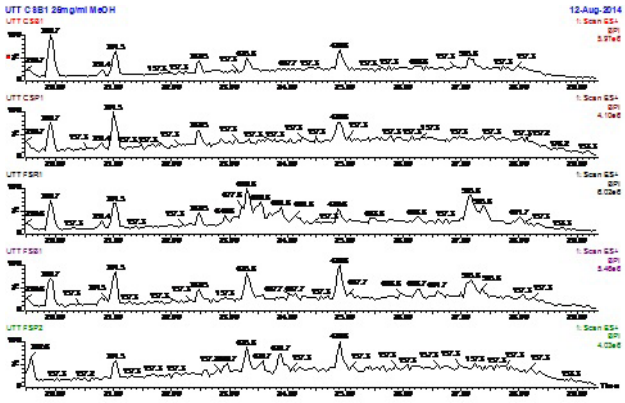

Figure 7. Chromatograms and associated spectra for ethanolic extracts of high fibre tree bark [CSB1], pulp [CSP1] and high fibre roots [FSR1], bark [FSB1] and pulp [FSP2] of *A. muricata*.

have a molecular weight [MW] of 500 or above (Figures 1 to 7). In several of the extracts of the CSP, CSR, and FSR samples, the acetogenins were absent, whereas in the other chromatogram group the same peaks can be identified as in the samples above (e.g. the common peaks with MW 304.6, 332.7 etc. plus some additional higher molecular weight compounds). Generally, the root extracts of the high fibre [FS] and fibreless [CS] fruits trees had more acetogenins than other plant parts, and the FS showed more peaks corresponding to the acetogenins.

These compounds are the acetogenins (i.e. those with molecular ion 635.8, and 585 MW) which were absent from the blank. The fibre free bark [CSB1 and CSB2] extracts (Figure 1) did not vary and both contained compounds with molecular ion 635 (probably 634 mw, if the molecular ion is M+H in positive mode MS, but there is no known compound in *Annona* with MW634). Similarly, the high fibre root [FSR1] extract (Figure 2) showed that there is a compound with M+H ion of 619 which could be a 618 acetogenin. However, there is no known acetogenin with MW 618.

A closer examination of the high fibre pulp [FSP] and roots [FSR] extracts (Figure 3) revealed an expansion of the 21-29 minute range showing a variety of peaks with MW similar to that expected for acetogenins 3,6,11. It was very difficult to confirm the presence of any other acetogenins as the MW for the peaks that looked like them do not match known or reported compounds. This suggests that such peaks may correspond to compounds not yet identified or characterized (Figures 4 to 7).

What is evident is that the high fibre bark [FSB], root [FSR] and fibre free bark [CSB] have similar compounds to CSP and CSR, but also have several additional compounds which are not yet identified. There is scope for additional analysis to isolate these compounds from larger quantities of material and to determine structures and it is not easy to determine that for acetogenins either.

Conclusions

Annona species have been used as a natural remedy for a variety of illnesses as it is assumed that it contains a number of bioactive compounds in the leaves, fruit, seeds, and stem [14] which may have healing properties. In profiling the characteristics of the two varieties of soursop in the Caribbean, the emphasis was on the ethno-medicinal value placed on the use of the fruit and other plant parts. There is also the notion that the teas made from the leaf is of significant folk-medicinal use, but this is still unconfirmed. The study has revealed that several new compounds exist, but were not previously identified as present in the pulp and other plant tissues from both varieties. Additional work is ongoing to isolate and positively identify these actogenins and complete the profile of these two varieties.

This study led to the recommendation of improving chemical characterization of both varieties and doing the biological evaluation of major components in order to identify the chemical components responsible for the biological activities of extracts. The scientific value and utility of the results of this work can propel further work in the phyto-chemistry of anti-cytotoxic compounds in tropical trees used in ethno-medicine.

Acknowledgements

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References

1. Janick J, Paul RE. 2008. Eds. The encyclopedia of tropical fruits and nuts. *CAB International*. 2008.

2. Campbell CW. Cultivation of fruit of the Annonacacae in Florida. *Proceedings of the American Society of Horticultural Science Tropical Region.* 1985; 18: 539-541.
3. Chang FR, Wu YC. 2001. Novel Cytotoxic Annonaceous Acetogenins from Annona muricata. *J. Nat. Prod.* 2001; 64 (7): 925–93.
4. Sousa OV, Vieira G DV, De Pinho J D J R, Yamamoto CH, Alves M S. Antinociceptive and anti-inflammatory activities of the ethanol extract of Annona muricata L. leaves in animal models. *International journal of Molecular Sciences.* 2010 ; 11(5): 2067-2078.
5. Heinrich M. Parasitological and Microbiological Evaluation of Mixe Indian Medicinal Planta (Mexico). *J Ethnopharmacol.*1992; 36 (1): 81-85.
6. Champy P, Melot A, Guérineau Eng V, et al. Quantification of acetogenins in Annona muricata linked to atypical parkinsonism in Guadeloupe. *J Nat Prod.* 2002 ;65(4):470-5.
7. McLaughlin J L. Paw Paw and cancer: Annonaceous acetogenins from discovery to commercial products. *Journal of Natural Products.* 2008;71(7):1311–21. doi:10.1021/np800191t. PMID 18598079.
8. Barbalho SM, Débora C, Spada APM, et al. Annona sp: Plants with multiple applications as alternative medicine. *Journal of Agricultural and Food Chemistry.* 2010; 28(10):571–574.
9. Sousa OV, Del-Vechio-Vieira G, Kaplan MAC. Propriedades analgésica e antiinflamatória do extrato metanólico de folhas de Annona coriacea Mart. (Annonaceae). *Lat. Am. J.* Pharm. 2007; 26: 872-877.
10. Alali FQ, Liu XX, McLaughlin JL. Annonaceous acetogenins: recent progress. *Journal of Natural Products.* 1999; 62(3): 504-540.
11. Rieser MJ, Gu ZM, Fang XP, et al. 1996. Five novel monotetrahydrofuran rings from seeds of Annona muricata. *J. Nat Prod.* 1996;59(2):100-
12. Zeng L, Wu FE, Oberlies NH, et al. 1996. Five new monotetrahydrofuran ring acetogenins from the leaves of Annona muricata. *Journal of Natural Products.* 1996; 59(11): 1035-1042.
13. Gholse YN, Yadav SR. Developments in nutraceuticals for chemoprevention. *International Journal of Pharmacy & Technology.* 2012; 4(1): 1950-1973.
14. Barbalho SM, Alvares Goulart RD, Farinazzi-Machado FV, et al. Plants with multiple applications as alternative medicine—A review. *Current Bioactive Compounds.* 2012; 8(3): 277-286.
15. Bridgemohan P, Bridgemohan RSH. Evaluation of decoction extracts of two types of soursop (Annona muricata) for annonaceous acetogenin properties. *Acta Hort.* 2014; 1047:213-220.
16. Yang H, Zhang N, Zeng Q, et al. 2010. HPLC method for the simultaneous determination of ten annonaceous acetogenins after supercritical fluid CO^2 extraction. *International Journal of Biomedical Science: IJBS.* 2010; 6(3): 202.
17. Green PW, Stevenson PC, Simmonds MS, Sharma HC. Phenolic compounds on the pod-surface of pigeonpea, Cajanus cajan, mediate feeding behavior of Helicoverpa armigera larvae. *Journal of Chemical Ecology.* 2003;29(4):811-821.
18. Stevenson PC, Kite GC, Lewis GP, et al. 2012. Distinct chemotypes of Tephrosia vogelii and implications for their use in pest control and soil enrichment. *Phytochemistry.* 78, 135-146.