



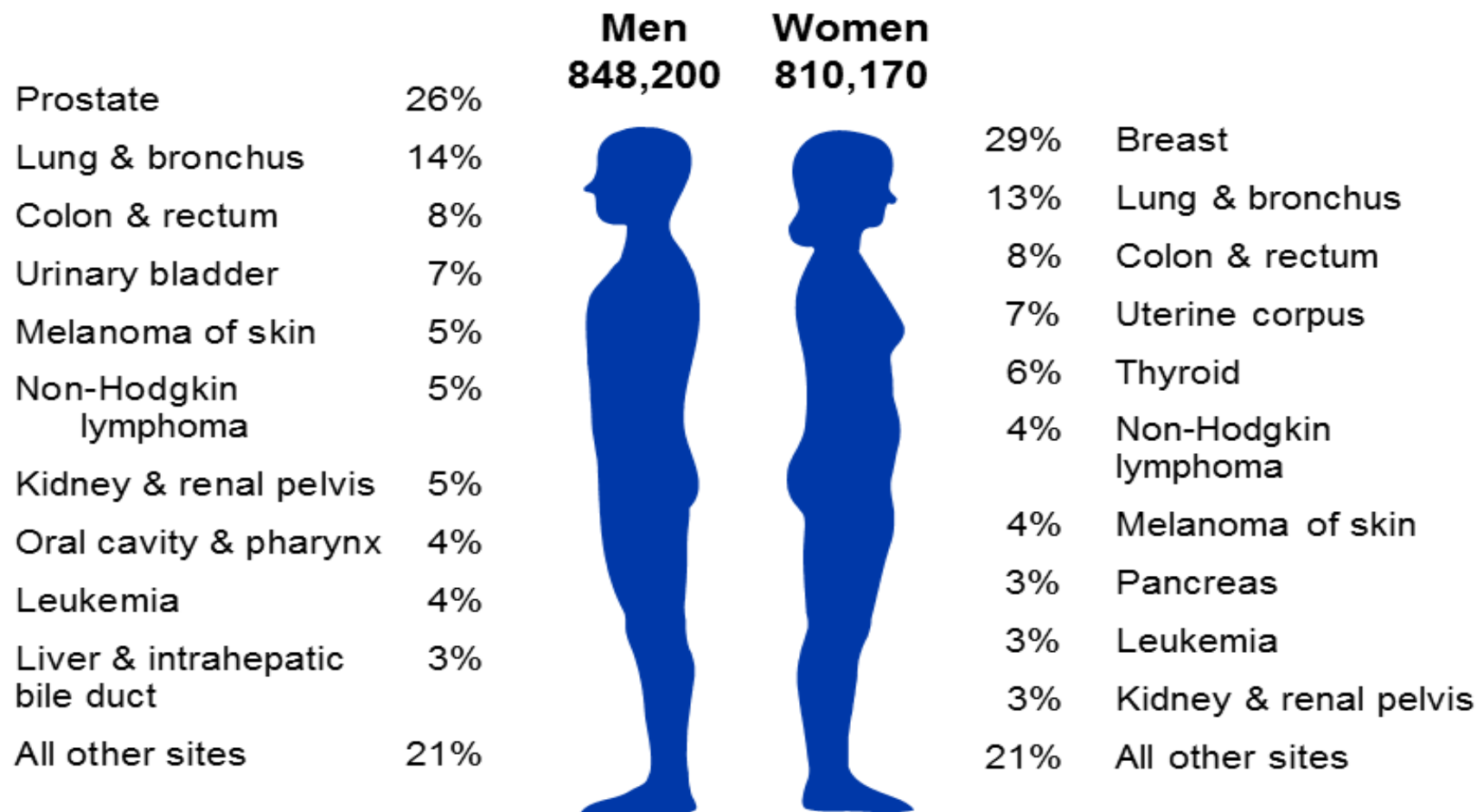
Acetogenin Update

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Disclaimer

- The following information is for educational purposes only and is not intended for the mitigation, cure or treatment of medical conditions.
- KSU does not endorse any products by any companies

Estimated New Cancer Cases* in the US in 2015



*Excludes basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.

The Lifetime Probability of Developing Cancer for Men, 2009-2011*

Site	Risk
All sites [†]	1 in 2
Prostate	1 in 7
Lung & bronchus	1 in 13
Colon & rectum	1 in 21
Urinary bladder [‡]	1 in 26
Melanoma of the skin [§]	1 in 34
Non-Hodgkin lymphoma	1 in 42
Kidney & renal pelvis	1 in 49
Leukemia	1 in 59
Oral cavity & pharynx	1 in 65
Pancreas	1 in 66

* For those free of cancer.

[†] All sites exclude basal cell and squamous cell skin cancers and in situ cancers except urinary bladder.

[‡] Includes invasive and in situ cancer cases

[§] Statistic for white men.

The Lifetime Probability of Developing Cancer for Women, 2009-2011*

Site	Risk
All sites†	1 in 3
Breast	1 in 8
Lung & bronchus	1 in 16
Colon & rectum	1 in 22
Uterine corpus	1 in 37
Non-Hodgkin lymphoma	1 in 52
Melanoma of the skin‡	1 in 53
Thyroid	1 in 60
Pancreas	1 in 67
Ovary	1 in 75
Leukemia	1 in 84

* For those free of cancer.

† All sites exclude basal cell and squamous cell skin cancers and in situ cancers except urinary bladder.

‡ Statistic for white women.

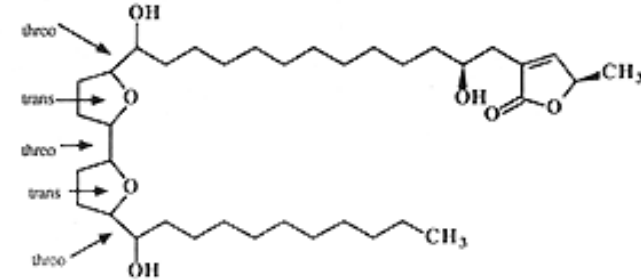
Acetogenin Compounds and Cancer: Research at Purdue University



- Dr. Jerry McLaughlin
 - Professor of pharmacognosy
- Extracts of pawpaw were are among the most potent of the 3500 species of higher plants screened for bioactive compounds in his laboratories
- <https://www.youtube.com/watch?v=hD6MGd0Dz5o>

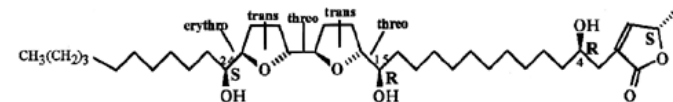
Pawpaw Acetogenin Compounds

- Annonaceous acetogenins
 - Long unbranched fatty acid chain
 - over 50 unique annonaceous acetogenins



Asimicin

- Inhibitor of complex I of the mitochondrial respiratory chain.
- Present in young pawpaw twig and other tissues



Bullaticin

Anti-tumor Activity

Table 1. The bioactivities of bullatacin in the brine shrimp test and human solid tumor cell lines.

Compound	BST LC ₅₀ (µg/ml)	ED ₅₀ (µg/ml)	MCF-7 ED ₅₀ (µg/ml)	HT-29 ED ₅₀ (µg/ml)
Bullatacin	1.59×10^{-3}	1.25×10^{-13}	>10	1.0×10^{-12}
Adriamycin	2.57×10^{-1}	1.04×10^{-4}	1.76×10^{-2}	1.53×10^{-4}

BST = brine shrimp lethality test

Adriamycin® = standard anticarcinoma drug

ED₅₀ = effective dose resulting in 50% cell death

LC₅₀ = lethal concentration resulting in 50% deaths

A-549 = human lung carcinoma

MCF-7 = human breast carcinoma

HT-29 = human colon carcinoma

Bullatacin inhibits mammalian solid tumor cells at concentrations often a billion times lower than Adriamycin., as shown in Table 1 (Zhao, et al. 1994b).

Pawpaw Acetogenin Compounds

- The powerful cytotoxicity, in vivo antitumor, pesticidal, antimalarial, antiviral, and antimicrobial effects indicated a myriad of potentially useful applications.
- Commercial development of these compounds
 - shampoo, highly effective in treating infestations of head lice, fleas, and ticks.
 - pesticidal sprays, which protects host plants against a diversity of pests.
 - an ointment for treatment of oral herpes (HSV-1) and other skin afflictions.
 - The extract (in capsule form) has been used by certain cancer patients as a botanical supplement product.

Home | Shop | Single Herbs | Paw Paw Cell-Reg™ (180 caps)

Paw Paw Cell-Reg™ (180 caps)

Stock No. 515-6



Paw Paw Cell-Reg selectively targets specific cells, modulating ATP production and blood supply to enhance the overall health of the body.

Retail Cost: \$68.55

Member Cost: \$45.70

Qty:

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Description

Label

Botany and Genetic Resources



■ *Asimina triloba* is a member of the tropical Custard Apple or Annonaceae family.

- custard apple (*Annona reticulata* L.)
- cherimoya (*A. cherimola* Mill.)
- sweetsop or sugar apple (*A. squamosa* L.)
- atemoya (*A. squamosa* x *A. cherimola*)
- soursop (*A. muricata* L.)



Consumption of Annona fruit and tea- A Link to Parkinsonism?

- Caparros-Lefebvre and Elbaz (1999) reported in Guadeloupe, atypical parkinsonism was abnormally frequent, and represented 75% of progressive parkinsonism while Parkinson's disease accounted for only 25% of the cases.
 - inversed percentage in comparison with patients living in Europe and North America.
- A higher proportion of patients with atypical parkinsonism (consumed soursop fruit 97%; consumed herbal tea 83%) than patients with Parkinson's disease (fruit 59%; herbal tea 18%) or control with no Parkinson symptoms (fruit 60%; herbal tea 43%).

Consumption of an Acetogen Compound-Annonacin

- Champy et al. (2005) determined the concentrations of annonacin, the major acetogenin in soursop.
 - An average fruit contains about 15 mg of annonacin.
- When directly injecting rats for one month with the estimated amounts of annonacin ingested in a year by eating one fruit daily, this induced neurodegeneration in the basal ganglia and mesencephalon (Champy et al., 2004).
- Purified extract and bioavailability upon digestion not examined.

Consumption of *Annona* fruit and tea-A Link to Parkinsonism?

- The prevalence of Parkinson's disease is roughly 150 per 100,000 inhabitants in the United States or Europe (Schrag et al., 1999).
- Caparros-Lefebvre et al. (2005) calculated that the number of PD cases in Guadeloupe would be approximately 640 (out of 420,000 people)
 - 152 per 100,000
 - Younger people who had symptoms and stopped consuming *Annona* products reversed their symptoms

Atypical Parkinsonism in Guadeloupe

- Sensitivity to acetogenin compounds may be limited to those with a specific genetic predisposition in Guadeloupe
- The H2 allele has been reported to carry variants that are protective PSP and is rare (5% vs. 20%) in the Guadeloupian population
 - may contribute, in association with unidentified genetic or environmental risk factors, to disease susceptibility (Camuzat et al., 2008).

Table 1. Brine shrimp lethality and percentage yields of F005 (90% aqueous methanol fraction) from various plant parts of *Asimina triloba*.

Plant part extracted	LC ₅₀ (ppm) ^z	Yield of F005 (%)
Twigs ^y	0.042 (0.02-0.09)	1.78
Unripe fruit ^x	0.060 (0.03-0.08)	5.11
Root wood ^w	0.060 (0.03-0.08)	1.27
Seed ^v	0.065 (0.03-0.10)	4.03
Stem bark ^{x,u}	0.077 (0.04-0.12)	2.71
Stem bark ^{y,u}	0.102 (0.06-0.15)	1.96
Stem bark ^y	0.104 (0.03-0.20)	2.52
Root bark ^w	0.135 (0.09-0.21)	2.72
Whole stem: wood, bark and leaves ^y	0.202 (0.14-0.31)	1.60
Stem wood ^y	4.86 (0.37-11.93)	0.90
Leaves ^y	53.6 (33-82)	4.36

Objective

To determine if extracts of ripe pawpaw fruit pulp display acetogenin activity.

Brine Shrimp Lethality Assay (BST)

- *Artemia saline* or Sea Monkeys
- Rapid, in house, and low cost substitute for cytotoxic assays developed by Dr. McLaughlin
- Responsible for the isolation of numerous, chemical and pharmacologically diverse antitumor and pesticidal compounds.
- Records the lethal concentration to kill 50% of the shrimp (LC50 value with upper and lower 95% confidence levels)

Brine Shrimp Test-Acetogenin Activity

- About nine grams of pawpaw pulp was extracted with ethanol and concentrated through a series of methanol and chloroform extractions as described by Ratnayake et al., (1993).
- Twenty brine shrimp larvae, taken 48 h after initiation of hatching in artificial sea water were added to each vial (5mL) containing 0, 10, 100 and 1000 ppm concentrations of extract.
- After 24 h, survivors were counted. SD from three extracts.

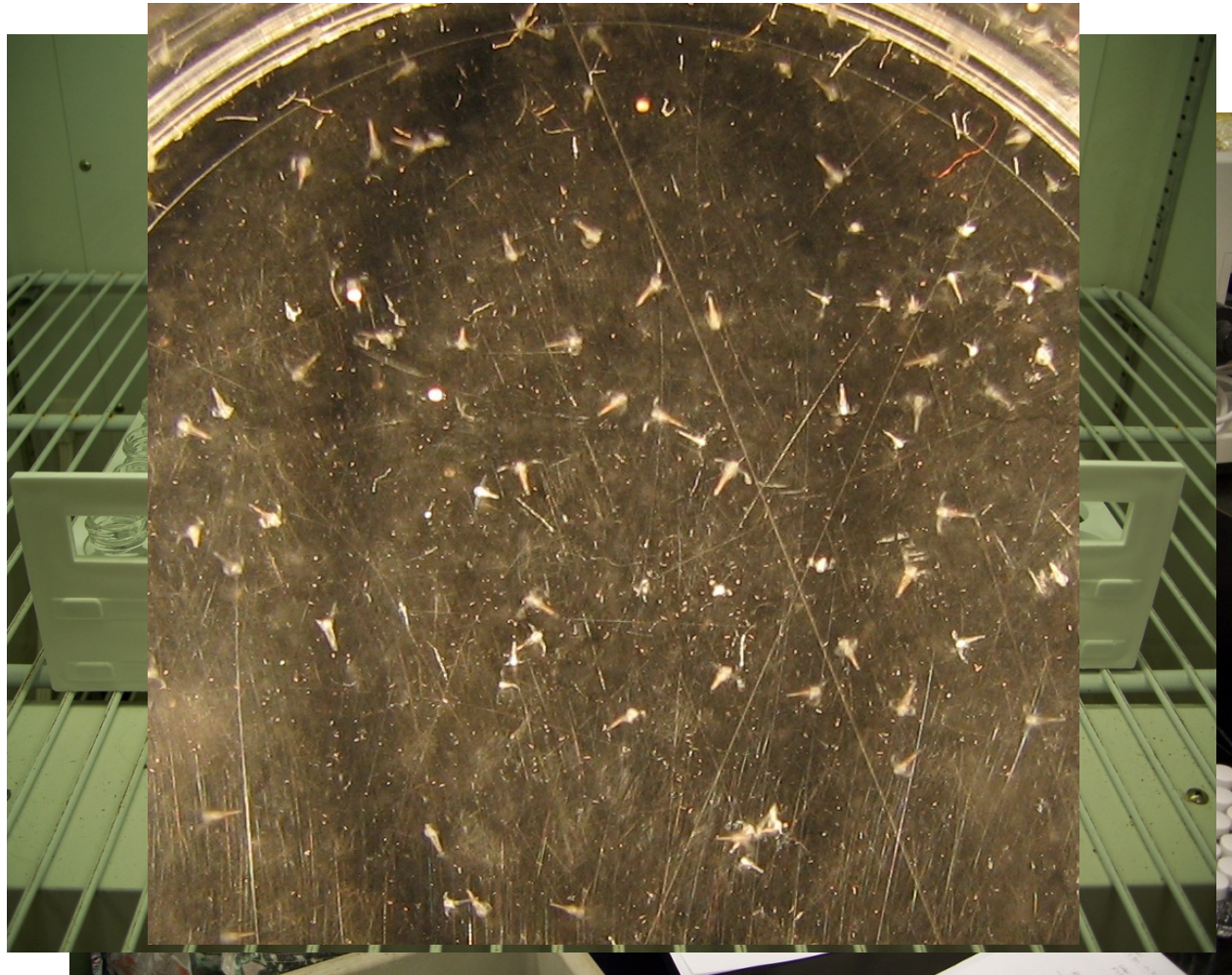
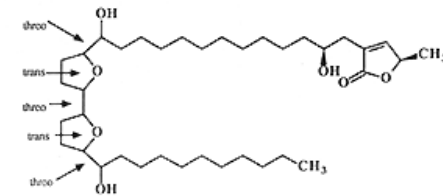
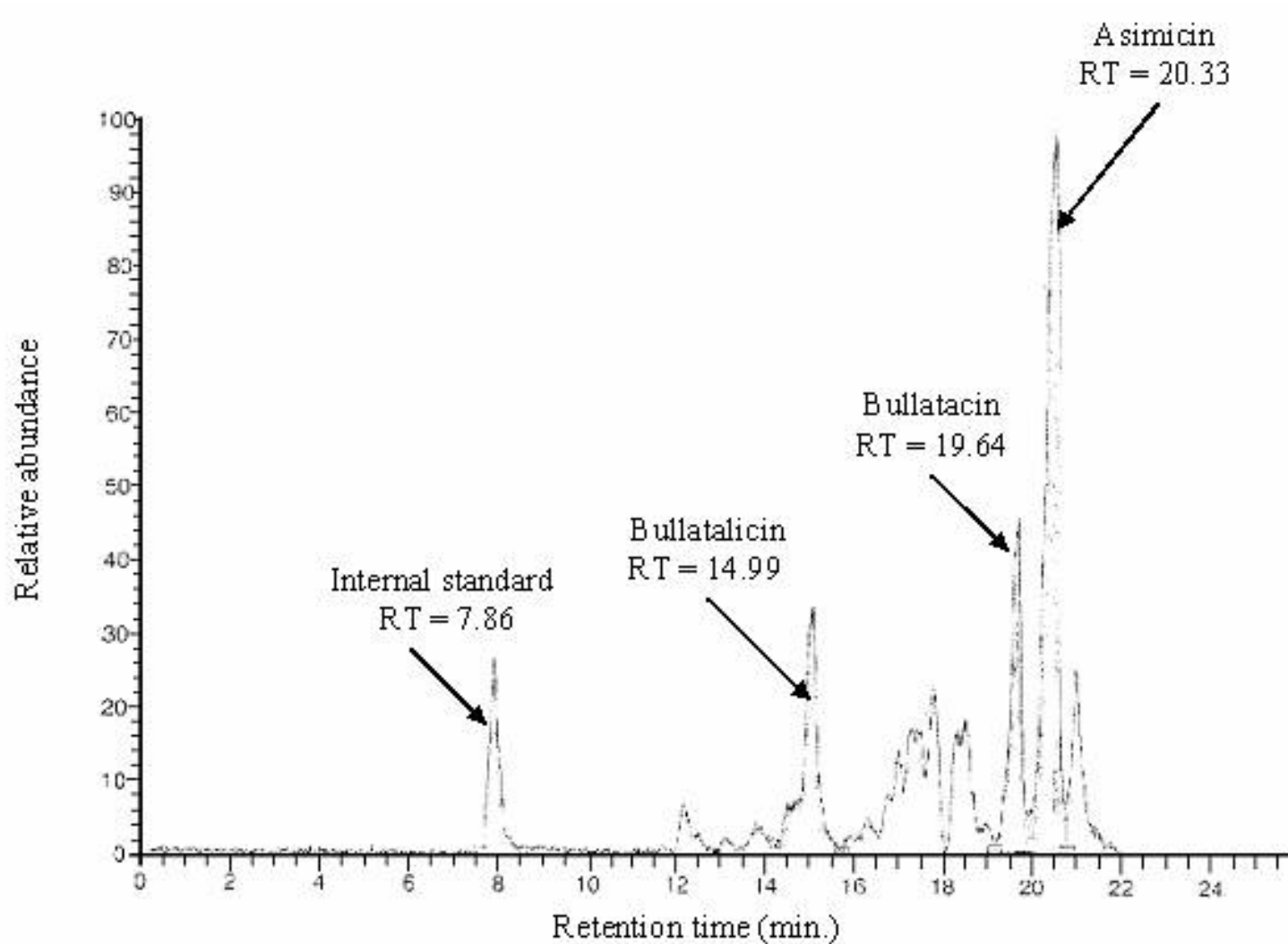


Table 3. Brine Shrimp Mortality (LC₅₀) Values for the Pawpaw Cultivars ‘Mitchell’, ‘NC-1’, ‘Overleese’, ‘Zimmerman’, ‘Wells’, and ‘Sunflower’ from Frozen Pulp of Ripe Fruit^a

pawpaw cultivar	LC ₅₀ (ppm)	pawpaw cultivar	LC ₅₀ (ppm)
‘Mitchell’	0.31 (±1.02)	‘Zimmerman’	4.09 (±0.49)
‘Overleese’	0.38 (±1.48)	‘Wells’	7.33 (±1.27)
‘NC-1’	0.89 (±1.41)	‘Sunflower’	8.60 (±0.42)

^a Parentheses enclose standard error.

Confirmation of Acetogenins in 'NC-1' Pulp Subjected to HPLS-Mass Spec Analysis



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Neurotoxicology. 2012 Jan;33(1):53-8. doi: 10.1016/j.neuro.2011.10.009. Epub 2011 Nov 23.

Annonacin in Asimina triloba fruit: implication for neurotoxicity.

Potts LF¹, Luzzio FA, Smith SC, Hetman M, Champy P, Litvan J.

⊕ Author information

Abstract

INTRODUCTION: The acetogenin, annonacin, from the tropical annonaceous plant *Annona muricata*, is a lipophilic, mitochondrial complex I inhibitor reported to be more toxic than rotenone to mesencephalic neurons. The temperate annonaceous plant *Asimina triloba* (pawpaw) is native to the Eastern United States and products are available online. This study determined whether annonacin is in the pawpaw fruit pulp and whether it or the crude ethyl acetate extract is toxic to cortical neurons.

METHODS: Pawpaw extract was prepared by pulp extraction with methanol and liquid-liquid partitioning with ethyl acetate (EtOAc). Annonacin was isolated from the crude EtOAc extract via column chromatography using a gradient solvent system of increasing polarity. Mass spectroscopy, nuclear magnetic resonance and infrared spectroscopy were used to compare isolated material with synthetic annonacin data and a natural annonacin sample. Toxicity of isolated annonacin and the total EtOAc extract was determined in primary rat cortical neurons using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

RESULTS: The average concentration of annonacin in the fruit pulp was 0.0701 ± 0.0305 mg/g. Purified annonacin (30.07 μg/ml) and crude EtOAc extract (47.96 μg/ml) induced 50% death of cortical neurons 48h post treatment. Annonacin toxicity was enhanced in the presence of crude extract.

DISCUSSION: Pawpaw fruit contains a high concentration of annonacin, which is toxic to cortical neurons. Crude fruit extract also induced neurotoxicity, highlighting the need for additional studies to determine the potential risks of neurodegeneration associated with chronic exposure to pawpaw products.

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J Agric Food Chem. 2015 Jan 22. [Epub ahead of print]

Determination of Neurotoxic Acetogenins in Pawpaw (*Asimina triloba*) Fruit by LC-HRMS.

Levine RA¹, Richards KM, Tran K, Luo R, Thomas AL, Smith RE.

+ Author information

Abstract

The concentrations of the neurotoxins, annonacin and squamocin, were determined in a lyophilized sample of the fruit pulp of the North American pawpaw (*Asimina triloba*) by LC coupled to high resolution mass spectrometry or LC-HRMS. The sample was extracted using dry methanol at 100 °C and 10 MPa pressure in a sealed container. The extraction of annonacin and squamocin was optimal at 100 °C with 7.72 and 0.162 mg/g, respectively, being found. Also, several isomers of annonacin and squamocin were separated and detected but not quantified.

KEYWORDS: *Asimina triloba*; LC-HRMS; Parkinson's disease; annonacin; pawpaw; squamocin

PMID: [25594104](#) DOI: [10.1021/jf504500g](#)

[PubMed - as supplied by publisher]





Journal of Chromatography B

Volume 1004, 1 November 2015, Pages 46–52



Method development for quantification of the environmental neurotoxin annonacin in Rat plasma by UPLC–MS/MS and application to a pharmacokinetic study

Natacha Bonneau^a, Isabelle Schmitz-Afonso^{b, 1}, Alain Brunelle^b, David Touboul^b, Pierre Champy^a,  

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- The bioavailability of annonacin was estimated to be $3.2 \pm 0.3\%$ of the ingested dose.
- The low bioavailability observed here offers insight into a low brain exposure to AAGs, comparatively to levels of dietary intake in Annonaceae consumers.

- “It gives clue to the absence of acute toxicity of alimentary products containing annonacin, and might justify the fact that Annonaceae fruits containing high levels of this cytotoxic compound (e.g. *Annona muricata*, *Asimina triloba*) reached an edible status in Human and are cultivated as commercially-valuable crops.”

What the FDA Says:

- Kentucky State University asked the U.S. Food and Drug Administration (FDA) for an opinion on this topic, and their conclusion was that pawpaw has a long history of food use and the FDA does not currently have any evidence that pawpaw is unsafe to eat.

Table 1. Highest and Lowest Brine Shrimp Test LC₅₀ Values of Extracts from 135 Individual Paw Paw Trees Growing at the WMREC Plantation^{a,b}

high producers		low producers	
tree sample	BST-LC ₅₀ (ppm)	tree sample	BST-LC ₅₀ (ppm)
4-11	0.033 ± 0.0010	2-32	32 ± 12
5-82	0.053 ± 0.015	1-19	12 ± 4.7
4-70	0.056 ± 0.0354	4-46	10 ± 0.55
2-88	0.063 ± 0.020	5-14	8.4 ± 2.5

^a Grand mean LC₅₀ value of 1.2 ppm ± 0.30 ppm. ^b Twig samples were collected, September 26, 1995, from trees planted in the spring of 1984.

Summary

- We are screening current pawpaw cultivars for acetogenin activity:
 - High: NC-1, Overleese, Mitchell, Middletown, Susquehanna,...
 - Low: Sunflower, Wabash, Potomac, Zimmerman, Wells,...
- In the KSU pawpaw breeding program, we are selecting both high and low acetogenin genotypes.

Questions?