

Anticancer Activity of Acetogenins from *Annona Muricata* Fruit

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ABSTRACT

Medicinal plants become very important in our days for their therapeutic benefits to humankind. It sustains human health, and it is commonly known as herbal medicines since ancient times. *Annona muricata* is a heart-shaped fruit that is consumed raw or as the fruit juice in the tropical area. *A. muricata* is used in traditional and alternative medicine to treat different ailments such as diabetes, hypertension, respiratory and skin illness, inflammation and cancer. *A. muricata* contains essential anticancer agents named acetogenins that play the significant role in various cancer types. Acetogenins are strong nicotinamide adenine dinucleotide oxidase inhibitors of the cancer cell's mitochondrial membrane but showed neurotoxic effects in rats. Therefore, acetogenins need to be further investigated to determine the exact mechanisms of action, long-term safety, optimal dosage, and potential side effects. Given the extensive studies on *A. muricata*, this review focuses on the phytochemistry, medicinal uses, biological activities and the mechanisms of action for the fruit extracts and acetogenins, to stimulate further studies on the fruit pulp used for human consumption.

KEYWORDS: Acetogenins, Anticancer, *Annona muricata*, complex I mitochondrial.

INTRODUCTION

Natural products from plants have been used for a considerable period by many cultures and civilizations to help humankind maintain its health and treat different diseases. Over the centuries, the phytochemical compounds have been an essential discovery in pharmaceutical applications. The significance of the active compounds from plants in medicine and agriculture has stimulated scientific interest in its biological properties.¹ In a pharmaceutical area, plants used in ethnomedicine are the most abundant source of active phytochemicals that afford health benefits against different ailments and diseases. One of it with many

traditional uses is *Annona muricata* L.² *A. muricata* is a species in the Annonaceae family that obtained much interested in the last decades due to its pharmaceutical potential. A long time ago, many studies reported on the medicinal use of the Annonaceae family, since then, the bioactivity and toxicity of this species attracted the attention of many researchers.^{2,3} Among the critical subject studied related to *A. muricata* is its acetogenins. This study will extensively collect relevant information regarding *A. muricata* acetogenins to identify the uncover information so that further investigation will be made.

BOTANICAL DESCRIPTION AND DISTRIBUTION

A. muricata is commonly known as soursop (English), graviola (Portuguese), guanabana (Latin; South America) and many other indigenous names. This plant species belongs to the genus of *Annona* and the Annonaceae family; the order of Magnoliales and Magnoliophyta Division.⁴ The *Annona* genus contains

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more than 70 species in which *A. muricata* is widely grown.³ *A. muricata* distributes in the warmest tropical region of Central and South America, Southeast Asia and Western Africa.⁴ It grows at altitudes below 1200 m above sea level, and temperatures between 25 and 28 °C, with humidity between 60 and 80% and annual precipitation over 1500 mm.



Figure 1. *Annona muricata* L fruit

A. muricata is a 4 to 6 meters high evergreen tree with large, glossy, dark green leaves.¹ Its edible fruits are large, heart-shaped, dark green in color,

with a diameter which varies between 5-20 cm and an average weight of 0.4 to 1.0 kg (Figure 1). Each fruit may include 55-170 black seeds.⁴ The pulp of the fruit has a sweet flesh, and typical flavor which can be eaten and used as an ingredient in the preparation of many foods and is perfect for making drinks, candy and ice cream. Also, the edible fruit, leaves, seeds, and root are known to have attractive medicinal properties.⁵

ETHNO MEDICINAL USE

All portions of *A. muricata* tree such as leaves, twigs, fruit, and seeds are used against many human ailments and diseases, particularly cancer and parasitic infection.⁶ The fruit is taken as herbal medicine to eliminate worms and parasites, for arthritic pain, diarrhea, neuralgia, dysentery, rheumatism, cold fever and to increase mother's milk after childbirth.⁷ Table I lists the ethnomedicinal use of *A. muricata* fruit.

Table I: Ethnomedicinal use of *A. muricata* fruit in the world

Worldwide ethnomedicinal use	Ethnomedicinal use	Preparation/ application	References
Bolivia	Kidney disorders, hypertension	Juice/oral	10
Brazil	Lactagogue, astringent, diarrhea, dysentery	Juice/oral	8
Colombia	Febrifuge, inflammation	Juice/oral	11
The Dominican Republic	Galactagogue	Infusion/oral	12
Haiti	Fevers, flu, heart affectation parasite, pellagra, anxiety, febrifuge, diarrhea, and lactagogue	NR	8
Jamaica	Fevers, parasites, diarrhea, and lactagogue	Juice/oral	13
Malaysia	Stomach pain, hypertension	Juice/oral	14
Mexico	Dysentery, diabetes	Juice/oral	15
The Philippines	Diabetes	Pulp/oral	8
Peru	Obesity, gastritis, dyspepsia, diabetes, inflammation, and cancer	Pulp, juice/ oral	8
Uganda	Diabetes	Pulp/oral	16
West Indies	Fevers, parasites, diarrhea, and galactagogue	Poultice/oral	13

Recently, *A. muricata* fruit consumption increased.¹¹ Researchers identified bioactive compounds such as acetogenins and polyphenols in its pulp, which are related to different pathology prevention including neurodegeneration, diabetes, cardiovascular and anti-inflammatory diseases.⁸ At present, the attention is being focused on anticancer properties of *A. muricata* acetogenins which are the primary bioactive compounds found in the fruit.⁹

PHYTOCHEMICALS

A. muricata flesh fruit consists of 80% water, 18% carbohydrates, 1% protein and small quantities of vitamins B, B2, C and dietary fiber.¹⁷ *A. muricata* fruit contains various significant minerals too, such as potassium (K), calcium (Ca), sodium (Na), copper (Cu), iron (Fe) and magnesium (Mg). Because of its nutritional values, Gymfi and co-workers (2011) suggested that the *A. muricata* fruit can provide essential elements and nutrients to the human body.¹⁸

Many phytoconstituents and compounds found in different parts of *A. muricata*, including alkaloids (ALKs), phenolics (PLs), megastigmanes (MGs), flavonol triglycosides (FTGs), cyclopeptides (CPs) and essential oils.¹ Additionally, *A. muricata* has shown to be the most abundant source of the annonaceous acetogenin compounds (AGEs), in which, researchers have identified more than 120 acetogenins from methanolic, ethanolic or another organic extract of different part of *A. muricata* such as leaves, seeds, bark, stems, pulp and fruit peel.^{20,21}

ANNONACEOUS ACETOGENINS

Acetogenins are secondary metabolites of C35/C37 deriving from the long chain of unsaturated fats in the polyketide pathway. A methyl-substituted α , β -unsaturated γ -lactone form them, which is usually combined with fatty acids with a two-propanol unit at C-2 (Figure 2).^{20,22}

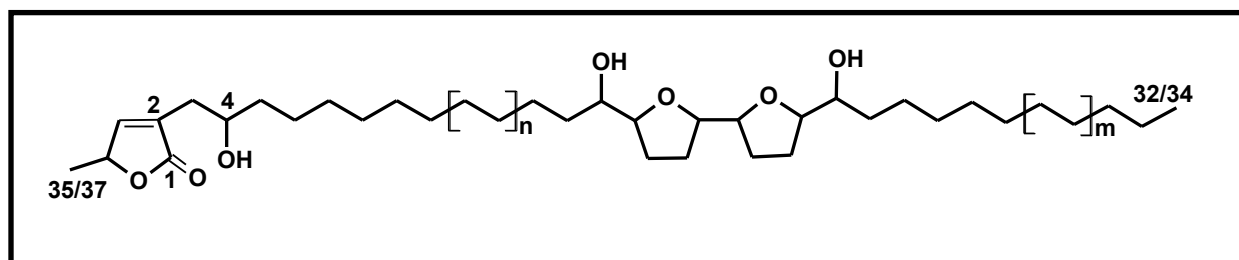


Figure 2: General structure of acetogenins.

AGEs are the main bioactive compounds of the Annonaceae family.²³ Since 1982 when Jolad et al. discovered uvaricin from *Uvaria accuminata*, more than 500 AGEs have been identified from various parts of plants in the Annonaceae family.²⁴ In recent years, many scientific studies focused on AGEs because of their unusual structures and extensive biological activities. Some studies have shown that AGEs are more cytotoxic than alkaloids and rotenone.³

The biological activities of AGEs, which are known as anticancer are initially by inhibiting the complex I mitochondrial of the cell (mitochondrial NADH:

ubiquinone oxidoreductase).²⁵ Biological studies and phytochemical investigations of the *A. muricata* fruit brought a wide range of AGE compounds, as summarized in Table II.

The chemical structures of the essential AGEs extracted from *A. muricata* fruit were shown in Figure 3. To the best of our knowledge, at the time of preparation of this paper (May 2018), at least 20 AGEs have been identified and isolated from the *A. muricata* fruit pulp.

Table II: Annonaceous acetogenins extracted from *A. muricata* fruit (AGE) and its biological activities.

Compounds	Stage of study	Biological Activity	Mechanism of action	References
Annonamuricin A, B, C, D	In vitro	Inhibition of human prostate cancer PC-3 cells	NR	9
Annonacin	In vitro	Inhibition of PC-3 cells	NR	9
Muricin M	In vitro	Inhibition of PC-3 cells	NR	26
Muricin N	In vitro	Inhibition of PC-3 cells	NR	26
Muricenin	In vitro	Inhibition of PC-3 cells	NR	26
Muricin J	In vitro	Inhibition of PC-3 cells	Inhibition of the complex I mitochondrial.	27
Muricin K	In vitro	Inhibition of PC-3 cells	Inhibition of the complex I mitochondrial.	27
Muricin L	In vitro	Inhibition of PC-3 cells	Inhibition of the complex I mitochondrial.	27
Annoreticuin-9-one	In vitro	Cytotoxicity against human pancreatic tumor (PACA-2), lung carcinoma (A-549) and prostate adenocarcinoma (PC-3) cell lines	NR	20
Cis-annoleticuin	In vitro	Cytotoxicity against human hepatoma cell line (Hep G2)	NR	20
Sabadelin	--	NR	NR	20
Epomuricenins-A	--	NR	NR	28
Epomuricenins-B	--	NR	NR	28
Epomusenin-A	--	NR	NR	28
Epomusenin-B	--	NR	NR	28
Epomurinin-A	--	NR	NR	28
Epomurinin-B	--	NR	NR	28

Anticancer Activity and Mechanism of Action of Acetogenins

Cancer is among the most common causes of morbidity and mortality in the world, about 1.73 million new cancer cases and 609,640 cancer deaths are projected to occur in the United States in 2018.²⁹ Many studies reported on the significance cytotoxicity of different extracts and isolated AGEs from a different part of *A. muricata* towards various cancer cell lines.^{30,31} However, few of these studies have illustrated or focus on the benefits of the fruit pulp against cancer disease. Mainly, the acetogenins extracted from the fruit pulp of *A. muricata* and its mechanism of actions.

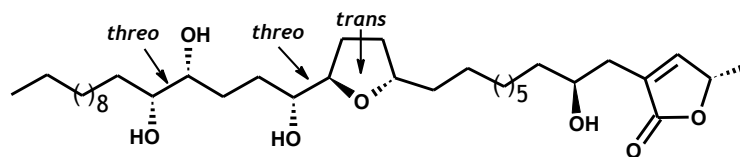
Previous data reported that *A. muricata* has an ability on growth inhibition against a wide variety of cancer cells, including solid breast tumor, lung carcinoma cell lines, prostate adenocarcinoma, colon adenocarcinoma cell lines. As well as on pancreatic carcinoma cell lines, human lymphoma cell lines, liver cancer cell lines and multi-drug resistant human breast adenocarcinoma.^{32,33}

Among many active ingredients, acetogenins modulate the production of mitochondrial adenosine triphosphate (ATP) of cancer cells. ATP reduces the nourishment of cancer cells by decreasing the growth of blood vessels. Previous studies have proposed that the mechanism of the cytotoxic action of acetogenins is the inhibition of the

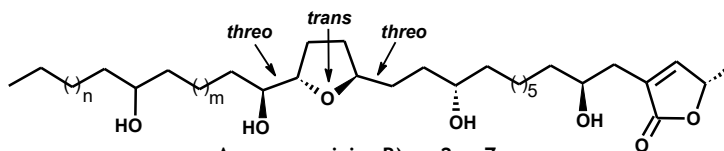
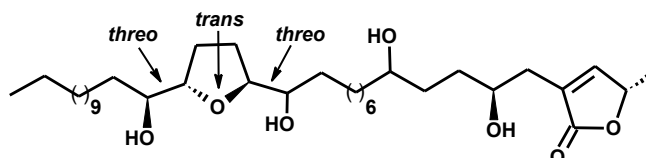
mitochondrial complex I by the inhibition of ubiquinone-linked NADH oxidase in the membrane causing apoptosis in cancer cells.^{34,35}

Complex I, the most complicated proton pump of the respiratory chain encoded by the nuclear and mitochondrial genomes, which is responsible for NADH oxidation, the hydrogenase of released electrons to electron acceptor ubiquinone and the proton translocation module.³⁶ Inhibition of

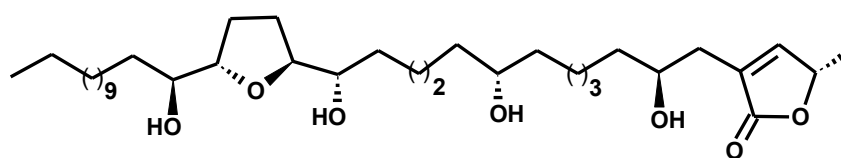
complex I diminish NADH oxidation which lowers the proton gradient through the internal mitochondrial membrane and the ATP synthesis. Moghadamtousi et al. have confirmed it by demonstrating that the extracts of *A. muricata* can arrest cancer cells development in G0/G1 phase which caused by the disruption of the mitochondrial membrane, and the induction of apoptosis to suppress the invasion and migration of cancer cells.³⁷



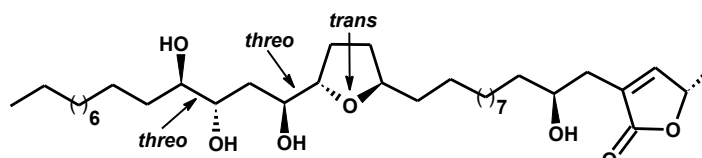
Annonamuricins A

Annonamuricins B) m=2 n=7
C) m=1 n=8

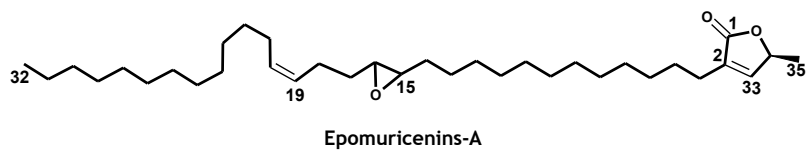
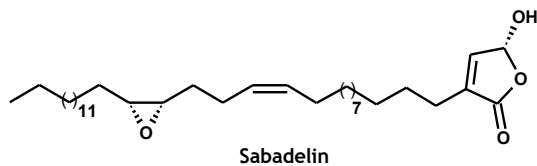
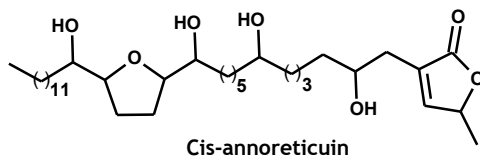
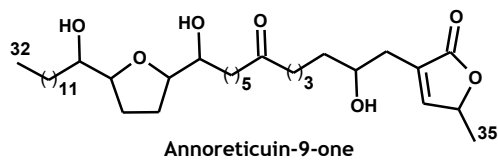
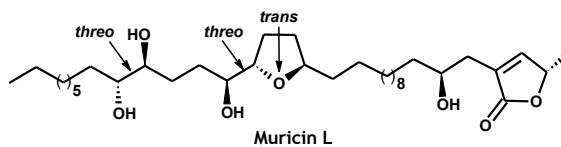
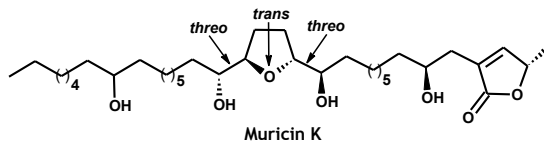
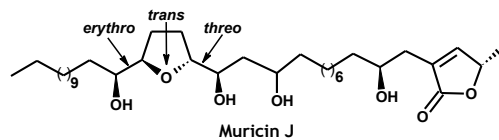
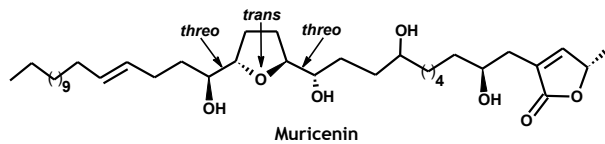
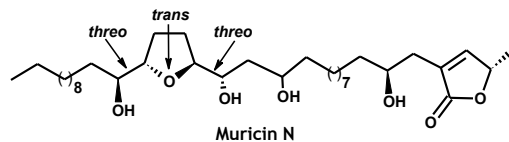
Annonamuricins D



Annonacin



Muricin M



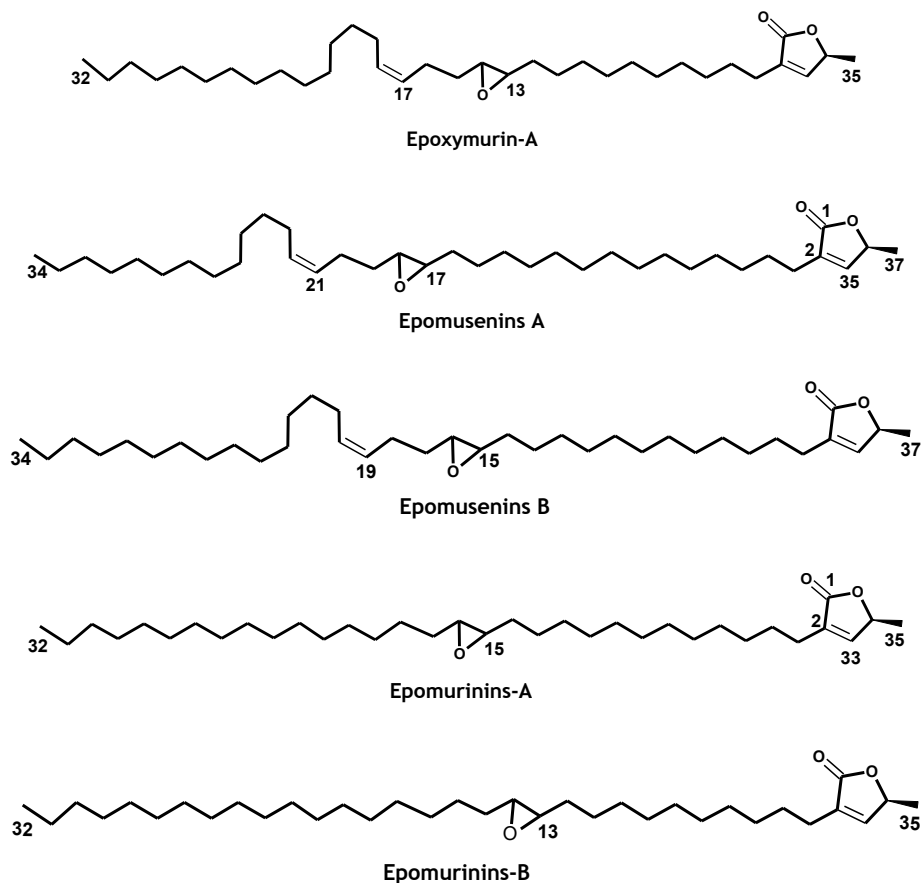


Figure 3: Chemical structures of the major acetogenins isolated from *A. muricata* fruit.

Moreover, the complex I dysfunction provoke the activation of the mitochondrial-dependent apoptotic machinery via triggering liberation of apoptogenic molecule cytochrome c from defective mitochondria.³⁶ Another study reported that Annonacin E caused depletion of mitochondrial membrane potential (MMP) which leads to the formation of apoptosome and the activation of caspase 9 and caspase 3/7, which have a relation with mitochondrial death pathway.³⁸ Additionally, Annonacin E upregulated Bax protein and down-regulates Bcl-2 proteins. This result confirms that Annonacin E caused apoptosis through the mitochondrial-mediated pathway. The AGEs apoptosis effect is selective for cancer cells is related to high ATP demand in cancer cells as suggested by McLaughling that selective cytotoxicity of *A. muricata* extracts is because of the enhanced ATP request of tumor cells compared to the healthy cells.³⁹

In vivo studies reported the anticancer activity of isolated acetogenins from *A. muricata* fruit reduced a breast tumor in rats when treated with *A. muricata* fruit extract for 5 weeks.⁴⁰ Many mechanisms of action of the cytotoxicity of *A.*

muricata were suggested including the inhibition of multiple signaling pathways, metastasis, cell cycle arrest and induction of necrosis.^{33,40} Ko and co-workers also reported that bullatacin extracted from *A. muricata* at doses of 0.4 g/kg could reduce a tumor induced in rodents 300 times better than the drug Taxol (paclitaxel).⁴¹ Meanwhile, Wang and co-workers reported that annonacin decreased tumour size in murine models at doses of 0.01 mg/kg compared to the commercial drugs such as adriamycin and cisplatin.⁴²

Although there are many AGEs isolated and identified from *A. muricata* fruit, there is insufficient understanding of its mechanisms of action in reducing tumor or cancer cells.^{20,21} Therefore, more study is needed to understand how AGEs work entirely. These significance anticancer and antitumor activities selected for *A. muricata* is due to the active compounds, especially AGEs that can be utilized as a cancer adjuvant treatment.³⁰

Study on Neurotoxin Effect of Acetogenins

The dysfunction of mitochondrial CI relates to a wide range of neurodegenerative diseases.³⁶ A published study in the Lancet Journal in 1999 investigated the

relationship between the consumption of *A. muricata* fruit and the occurrence of atypical parkinsonian syndromes observed in the French West Indies on 87 patients.⁴³ Furthermore, the etiology of that disease in Guadeloupe Island showed a close correlation between acetogenins consumption and appearance of the neurodegenerative illness.⁴⁴ A recently published study reported that annonacin as a significant AGE in *A. muricata* might be a potential risk factor for neurodegenerative diseases.⁴⁵ In rodent striatal neurons, annonacin minimized the ATP supply and interrupted the mitochondrial transportation to the cell soma; this caused perturbations in the protein tau which led to similar characteristics of neurodegenerative diseases.⁴⁴ It is anticipated that if somebody consumes one fruit of *A. muricata* daily, following one year, the aggregate amount of annonacin that has ingested is adequate to induce brain lesions in rats.⁴⁶

However, the number of the human population studied and the study in the rat may not represent a broader community of human in another area or continents. Because *A. muricata* is not new to those in warm tropical regions and a study by Muangpaisan and co-workers showed that the prevalence of Parkinson's diseases in Asian countries was slightly lower than that in Western nations, although *A. muricata* consumption is a routine practice among this people.⁴⁷ Despite some *in vitro* and *in vivo* studies reported on its neurotoxic compounds, Awodele and co-workers demonstrated that consumption of *A. muricata* fruit for 60 consecutive days did not induce any significant toxicity in a rat model.⁴⁸ Therefore, an in-depth study on the mechanism of action *in vitro* and *in vivo* and optimize consumption dosage at a specific AGEs must be carried out.

CONCLUSION

A. muricata fruit, an indigenous medicinal plant used for ages to treat many diseases. Many types of research have proven that *A. muricata* possesses a wide range of biological activities, and the most likely are anticancer, antidepressive and antiparasitic activity. However, there are limited studies on *A. muricata* phytoconstituents and compounds, especially acetogenins reported on its cytotoxic to the cancer cells especially on its mechanism of actions. Nevertheless, it is also said to have a neurotoxic effect.

More research is needed to identify AGEs mechanism of actions and quantify the neurotoxic molecules effects and determine the toxicity level to human. Finally, more clinical studies are required to verify and validate the plant extracts safety, to be adopted as a therapeutic anticancer agent.

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