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Review- Annona muricata as a cancer killer fruit

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Abstract
Cancers figure among the leading causes of morbidity and mortality worldwide, with approximately 8.2 million cancer related deaths in 2012 and is expected to rise by about 70 per cent over the next two decades. Cancer is the disease that commonly believed to be preventable. Chemoprevention of cancer can be defined as the use of natural, synthetic or biological substances that intervene in the early precancerous stages. Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine. These plants are rich sources of active phyto-constituents that provide medicinal or health benefits against various ailments and diseases. *Annona muricata i*t is a typical tropical tree with heart shaped edible fruits and widely distributed in most of tropical countries. Two hundred and twelve bioactive compounds have been reported to be found in *A. muricata*. The predominant compounds are acetogenins followed by alkaloids and phenols. All portions of the *A. muricata* tree are extensively used as traditional medicines against an array of human ailments and diseases, especially cancer and parasitic infections. Plenty of studies report the significant antiproliferative effects of different extracts of the plant and isolated AGEs (annonaceous acetogenin compounds) towards various cancer cell lines.

Keywords: *Annona Muricata*, Cancer, Nutritive facts and anti-hyperglycemic.

Introduction
Cancer is the uncontrolled growth of cells, which can invade and spread to distant sites of the body. Cancer figure among the leading causes of morbidity and mortality worldwide.
Among men, the 5 most common sites of cancer diagnosed in 2012 were lung, prostate, colorectum, stomach, and liver cancer. Among women the 5 most common sites diagnosed were breast, colorectum, lung, cervix, and stomach cancer. Cancer is a leading cause of death group worldwide and accounted for 8.2 million deaths (around 13% of all deaths) in 2014. The main types of cancer are: Lung (1.3 million deaths/year), stomach (803,000 deaths), colorectal (639,000 deaths), liver (610,000 deaths) and breast (519,000 deaths). More than 70% of all cancer deaths occurred in low- and middle-income countries. Deaths from cancer worldwide are projected to continue rising, with an estimated 11.5 million deaths in 2030 (WHO 2014).

Annona muricata
Medicinal plants are considered as the basis for health preservation and care worldwide. Chronic degenerative diseases (diabetes, cardiovascular and cancer) have reached epidemic proportions and are considered as a serious health problem; therefore, the treatments of these diseases are of clinical importance (WHO, 2005). *Annona muricata L.* is a species of the Annonaceae family that has been widely studied in the last decades due to its therapeutic potential. The medicinal uses of the Annonaceae family were reported long time ago (Gbaguidi, B. A., 2017) [2] and since then, this species has attracted the attention due to its bioactivity and toxicity.
Annonaceae, the custard apple family is a family of flowering plants consisting of trees, shrubs, or rarely lianas. With about 2300 to 2500 species and more than 130 genera, it belongs to the genus *Annona* and the family are concentrated in the tropics, with few species found in temperate regions. About 900 species are Neotropical, 450 are Afrotropical, and the other species Indomalayan. Guyabano tree, or soursop in English (Scientific Name: *Annona muricata Linn.*) is ethno medicinally important species from this family. Guyabano is

adaptable to tropical climate and are currently cultivated for its fruit in most Southeast Asian countries such as Malaysia, Indonesia and Philippines.

Nutritional facts of *annona muricata*

Table 1

Nutritional facts per 100 g	Contents
Energy	104.0 kcal
Total fat	0.3 g
Sodium	14 mg
Potassium	**278 mg**
Carbohydrate	23.5g
Dietary fiber	3.3 g
Protein	1.6 g
Riboflavin	170.0 µg
Vitamin C	**37.0 mg**
Calcium	17.0 mg
Phosphorus	47.0 mg
Carotene	120.0 µg
Thiamin	70.0 µg
Iron	1.5 mg

Yang, 2015

Phytochemicals

Two hundred and twelve bioactive compounds have been reported to be found in *A. muricata*. The predominant compounds are acetogenins followed by alkaloids, phenols and other compounds. Leaves and seeds are the main plant organs studied, probably because they are the most traditionally used. The majority of phytochemicals have been identified from organic extract, but recently focus has also been directed toward aqueous extracts. Several other compounds such as carbohydrates and essential oils have also been reported.

Alkaloids

Alkaloids are naturally occurring compounds containing basic nitrogen atoms. The most abundant in *A. muricata.*. A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms have shown that alkaloids isolated from Annona species possess an affinity for the 5-HT1A receptors in vitro and participate in dopamine biosynthesis (Hasrat *et al*., 1997) [3]. Thus, it has been proposed that alkaloids derived from the Annona could induce antidepressant-like effects and cytotoxic activity (Matsushige *et al*., 2012) [6]. Neurotoxic effects have also been reported for some alkaloids, and suggested that neuronal death occurred by apoptosis (Lannuzel *et al*., 2002) [4].

Phenolic compounds

Thirty-seven phenolic compounds have been reported to be present in *A. muricata*. The important phenolic compounds found in *A. muricata* leaves include quercetin and gallic acid. The presence of flavonoids and lipophilic antioxidant compounds such as tocopherols and tocotrienols has been reported to be present in the pulp (Correa-Gordillo *et al.*, 2012) [1]. In different studies, when organic or aqueous extracts have been used, the quantity of extractable total phenols is considerably different. This is important to mention because the most common medicinal use is aqueous infusion and the majority of phenols are soluble in water. Phenolic compounds are considered as the major phytochemicals responsible for the antioxidant activity (George *et al*., 2014).

Anticancer

a) Pancreatic cancer

The extract from *Annona muricata*induced necrosis of pancreatic cancer (PC) cells by inhibiting cellular metabolism. The expression of molecules related to hypoxia and glycolysis in PC cells (i.e. HIF-1α, NF-κB, GLUT1, GLUT4, HKII, and LDHA) were down-regulated in the presence of the extract. *In vitro* functional assays further confirmed the inhibition of tumorigenic properties of PC cells. Overall, the compounds present in the whole extract inhibited multiple signaling pathways that regulate metabolism, cell cycle, survival, and metastatic properties in PC cells.

The presence of Annonaceous acetogenins in the extract was evident bythe depletion of ATP production in PC cells. Current studies are undergoing to ensure that the cytotoxic effects are specific to tumorigenic cells only, by including the non-transformed immortalized pancreatic epithelial cell line HPNE, which is derived from pancreatic duct.

Results and Discussion

Annonacin, a mono-tetrahydrofuran acetogenin obtained from the seeds of *A.muricata* showed a significant cytotoxic effect on the T24 bladder cancer cells. Furthermore, annonacin activated p21 in a p53-independent manner and arrested T24 cells at the G1 phase. These results suggest that annonacin is potentially a promising anti-cancer compound. (Shyng *et al.*, 2003) [8].

The cytotoxicity of annonacin on cancer cells was further analyzed by morphologic changes (Fig. 1) and cell survival assay (Fig. 2), using T24 bladder cancer cells. At the dosage of 10 Ag/mL, nnonacin induced extensive apoptosis and cell detachment at 24 hrs after treatment (Fig. 1). Besides, the cell survival assay showed that only 56.0, 34.2, and 35.3% of the T24 cells were alive at 24, 48, and 72 hrs, individually, after annonacin treatment at the dosage of 1 Ag/mL, in comparison to an untreated control (Fig. 2).

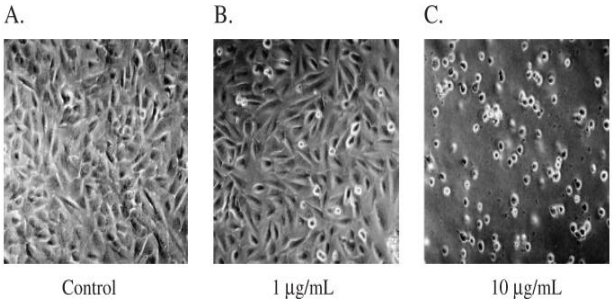


Fig 1: Morphologic changes of T24 cells after annonacin treatment for 24 hrs at the dosages of 0, 1, and 10 µg/mL.

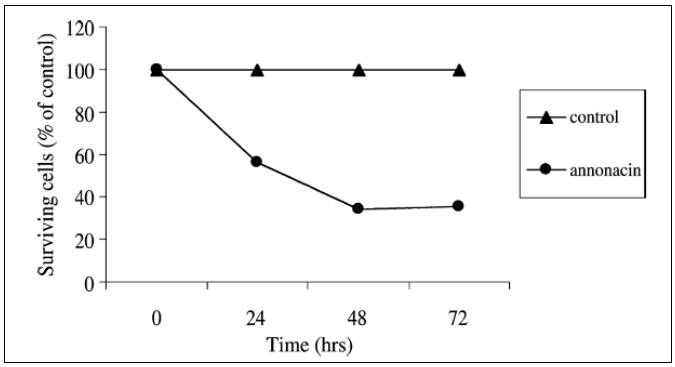
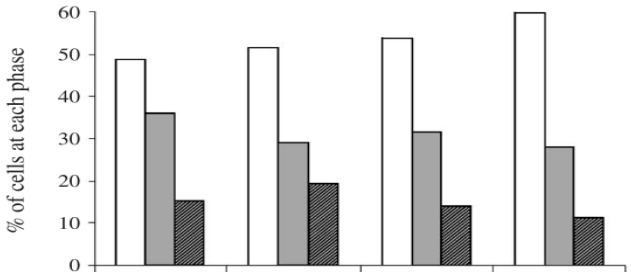


Fig 2

Fig. 2. Cell survival assay showing the percentage of surviving T24 cells after annonacin treatment. T24 cells were treated with 1 µg/mL annonacin for 24, 48, or 72 hrs, and the surviving cells were determined and presented as a percentage of the untreated cells (control).

To study the effect of annonacin on cell cycle progression, T24 cells were treated with annonacin for 24 hrs and the cell cycle distribution was determined by flow cytometry. Annonacin arrested T24 cells at the G1 phase in a dosage-dependent manner **(**Fig. 3). At the dosage of 1 Ag/mL, the G1 phase cells were Fig. 3.



	0 µg/mL	0.01 µg/mL	0.1 µg/mL	1 µg/mL
G0/G1	48.8	51.6	54.0	59.9
S	36.0	29.2	31.8	28.4
G2/M	15.2	19.2	14.2	11.6

Fig 3: Annonacin arrested T24 cells at G1 phase. T24 cells were treated with annonacin at different dosages for 24 hrs and the cell cycle distribution was determined by flow cytometry.

Yumin *et al.* (2011) [11] investigated the selective growth inhibition of human breast cancer cells (MDA-MB-468 cells) by graviola fruit extract (GFE) *in vitro* and *in vivo*. The results showed that the dietary GFE inhibited tumor growth, as measured by wet weight, by 32 per cent. Dietary GFE induced significant growth inhibition of MDA-MB- 468 cells *in vitro* and *in vivo* through a mechanism involving the EGFR/ERK (Epidermal Growth Factor Receptor/Extracellular Signal-regulated Kinase) signaling pathway, suggesting that GFE may have a protective effect for women against EGFR-over expressing breast cancer.

Suyatmi *et al.* (2012) [9] evaluated the cytotoxic selectivity of ethanolic extract of annona muricata leaves against HeLa cervical cancer cells and Vero cells line. The microscopic evaluation of the dead cells treated with 97µg/mL of extract showed numerous dead cells of HeLa cell but none of Vero cells. This confirms the selectivity of the extract against cell proliferation.

Results and Discussion MTT Assay

The cytotoxicity of ethanolic extract of *Annona muricata* leaf on HeLa and Vero cells is shown in Figure 4. The IC50 of the extract against HeLa cells was 97 µg/ml, while the value against Vero Cell line was 356 µg/ml.

Etidium Bromide-Acridin Orange Double Staining

The cytotoxic effect of the extract was visualized in Figure 5 and 6**.** The dead cells were stained as orange while the viable cells were stained green. The figure indicates the cytotoxic effect of the extract on HeLa cells. On the other hand, the activity was not observed in Vero cells line.

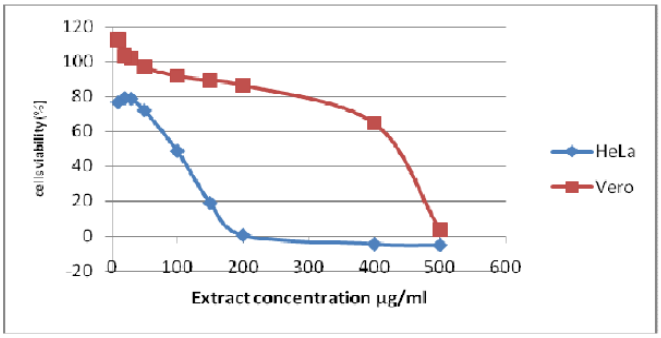


Fig 4: The cytotoxic effect of ethanolic extract of *Annona muricata* leaf.

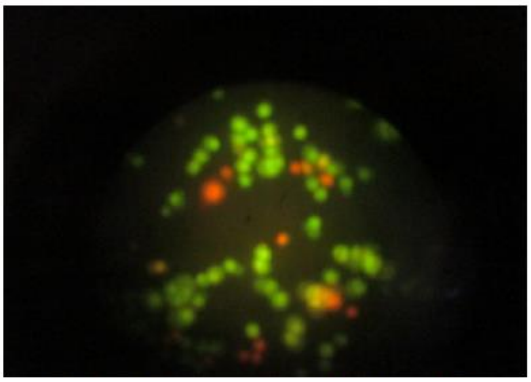


Fig 5: Double staining of HeLa cell treated with IC50 concentration of extract (97 µg/ml). The orange stained cells are the dead cells., while the green stain cells alive

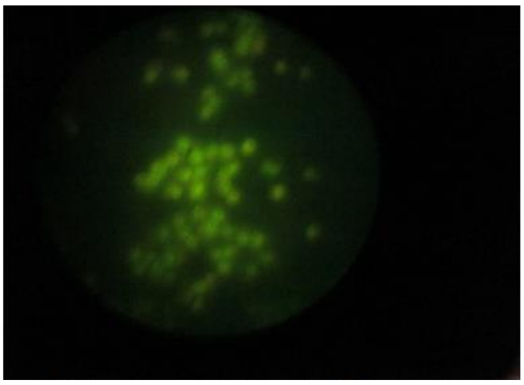


Fig 6: Double staining of HeLa cell treated with IC50 concentration of extract. All cells stains green, ne dead cells observed in Vero cells treated with the extract.

Discussion

Acetogenin is the active compound of *Annona muricata* leaf that kills the cancer cell through inhibition of NADH production. Previous studies has revealed a potent cytotoxic activity of this isolate on cancer cells line. In this research we observed the cytotoxic activity of the crude extract of *Annona muricata* leaf (Liu, *et al*., 2007; McLaughlin, 2008) [5]. Although the IC50 value calculated for the extract was quite high compare to the IC50 value of acetogenin isolate found in other research, this finding indicated a possibility to use crude extract as an alternative therapy for cervical cancer. The cytotoxic selectivity of the extract was also shown in our data. We calculate a high IC50 value of the extract (356 µg/ml)

against Vero cells proliferation. The microscopic evaluation of the dead cells treated with 97 µg/ml of extract using ethidium bromide-acridin double staining method showed numerous dead cells of HeLa cells but none of Vero cells. This data confirmed the selectivity of the extract against cancer cell proliferation.

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Conclusion

Cancer research is ongoing on these important plants and plant chemicals, as several pharmaceutical companies and universities continue to research, test, patent, and to synthesize these chemicals into new chemotherapeutic drugs. However, only a small proportion has been investigated both phytochemical and pharmacologically. There are gaps in the studies, which need to be bridged in order to exploit the full medicinal potential of *A. muricata*. This plant also has widespread use with extraordinary medicinal potential which should be better explored to find new biological properties which may increase its importance as efficient medicinal plant in biodiversity.

References

1. Correa Gordillo J, Ortiz D, Larrahondo JE, Sánchez Mejía M, Pachon H. Actividad antioxidante en guanábana (Annona muricata L.): una revisión bibliográfica. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*. 2012, 11(2).
2. Gbaguidi BA, Adjou ES, Koutchiko A, Dahouenon-Ahoussi E, Sezan A. Dominique S. Phytochemical and acute toxicity of ethanolic extract from leaves of *Annona muricata* (L.) from Benin in experimental albino rats. IJCS. 2017; 5(6):39-41.
3. Hasrat JA, Bruyne TD, Backer JP, Vauquelin G, Vlietinck AJ. Cirsimarin and cirsimaritin, flavonoids of Microtea debilis (Phytolaccaceae) with adenosine antagonistic properties in rats: leads for new therapeutics in acute renal failure. Journal of pharmacy and pharmacology. 1997; 49(11):1150-1156.
4. Lannuzel A, Höglinger GU, Champy P, Michel PP, Hirsch EC, Ruberg M. Is atypical parkinsonism in the Caribbean caused by the consumption of Annonacae?. In Parkinson's disease and Related Disorders. 2006, 153-157.
5. Liu HX, Huang GR, Zhang HM, Wub JR, Yaoa ZJ. Annonaceous acetogenin mimics bearing a terminal lactam and their cytotoxicity against cancer cells. Bioorg. Med. Chem. Lett. 2007; 17:3426-3430.
6. Matsushige A, Kotake Y, Matsunami K, Otsuka H, Ohta S, Takeda Y. Annonamine, a new aporphine alkaloid from the leaves of Annona muricata. Chemical and Pharmaceutical Bulletin. 2012; 60(2):257-259.
7. Santhosh GK, Dongmo, Moukette MS, Boyoum BM, Ngogang JY, Saxena AK. Antiproliferative activity and induction of apoptosis by *Annona muricata* (Annonaceae) extract on human cancer cells. BMC Complementary and Alternative Medicine. 2014; 14:516.
8. Shyng Y, Chang H, Chen H, Yeh Y, Kao Y, Lin K, *et al*. Annonacin, a mono-tetrahydrofuran acetogenin, arrests cancer cells at the G1 phase. Life Sciences, 2013; 72:2853-2861.
9. Suyatmi, Yuliana HS, Sinu AJ. The Selective Cytotoxicity of Ethanolic Extract of *Annona muricata* Leaf on HeLa Cervical Cancer Cells. Traditional Complementary and Alternative Medicine in Health Care. 2012; 30:237-244.
10. Yang C, Gundala SR, Mukkavilli R, Vangala S, Reid MD, Aneja R. Synergistic interactions among flavonoids and acetogenins in Graviola (*Annona muricata*) leaves confer protection against prostate cancer. Carcinogenesis, 2015; 36(6):656-665.
11. Yumin D, Hogan S, Schmelz ME, Canning C, Zhou K. Selective growth inhibition of human breast cancer cells by graviola fruit extract in vitro and in vivo involving down regulation of EGFR expression. Nutrition and cancer. 2011; 63(5):795-801.