DOI: 10.21276/ijabpt.2016.7.4.20

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-4, Oct-Dec-2016 Coden IJABFP-CAS-USA Copyrights@2016

Received: 6th Sept 2016 *Revised: 18th Oct 2016* *Accepted: 20th Oct 2016*

DOI: 10.21276/Ijabpt, http://dx.doi.org/10.21276/ijabpt *Research article*

ISOLATION AND IDENTIFICATION OF ACETOGENIN FROM *ANNONA MURICATA* LEAVES

Vinothini R[1] and Lali Growther[2]

Research Scholar[1] and Associate Professor and Head[2]
Department of Microbiology, Hindusthan College of Arts & Science, Coimbatore-6410 28.

ABSTRACT: *Annona muricata* is a member of the family Annonaceae. It is a variety of custard apple tree and species of the genus *Annona* known mostly for its edible fruit Annona. It commonly called as Graviola, soursop, Mullu seetha. Annonaceous acetogeneins are powerful phytochemicals found in the Graviola plant (*Annona muricata*), which are found only in Annonaceae family. These acetogenins are non-toxic to normal cells, but are highly toxic to cancer cells. These compounds, collectively, have shown antitumor, parasiticidal, pesticidal, and antimicrobial activities. The aim of this study was carryout for identify acetogenin from leaves of *Annona muricata* by using kedde's reagent and isolation of acetogenin fraction by open column chromatography. These findings support the traditional use of *Annona muricata* in varies disorders.

Key words: *Annona muricata*, acetogenin, kedde's reagent, thin layer chromatography, column chromatography.

*Corresponding author: Vinothini R, Department of Microbiology, Hindusthan College of Arts & Science, Coimbatore-6410 28, India, Email: vinothinirajan93@gmail.com

INTRODUCTION

A. muricata L., commonly known as soursop, graviola, guanabana, paw-paw and sirsak, is a member of the Annonaceae family comprising approximately 130 genera and 2300 species (Mishra et L., 2013). *A. muricata* is native to the warmest tropical areas in South and North America and is now widely distributed throughout tropical and subtropical parts of the world, including India, Malaysia and Nigeria, Australia, Africa (Adewole et al., 2006). *A. muricata* is an evergreen, terrestrial, erect tree reaching 5–8 m in height and features an open, roundish canopy with large, glossy, dark green leaves.

Many active compounds and chemicals have been found in graviola, as scientists have been studying its properties since the 1940s. Most of the research on graviola focuses on a novel set of chemicals called Annonaceous acetogenins. Graviola produces these natural compounds in its leaf and stem, bark, and fruit seeds. Three separate research groups have confirmed that these chemicals have significant antitumorous properties and selective toxicity against various types of cancer cells (without harming healthy cells). These groups have published eight clinical studies on their findings (Zeng, 1996). Many of the acetogenins have demonstrated selective toxicity to tumor cells at very low dosages as little as 1 part per million. acetogenins in graviola that are demonstrating the strongest anticancerous, antitumorous, and antiviral properties (Padma, 2001). Annonaceous acetogenins are only found in the Annonaceae family (to which graviola belongs). These chemicals in general have been documented with antitumorous, antiparasitic, insecticidal, and antimicrobial activities.

Mode of action studies in three separate laboratories have recently determined that these acetogenins are superb inhibitors of enzyme processes that are only found in the membranes of cancerous tumor cells. Acetogenins are characterized by a long aliphatic chain of 35 to 38 carbons bonded to a c-lactone ring, terminally substituted by b-unsaturated methyl (sometimes it is a ketolactone), with one or two Tetrahydrofurans (THF) located along the hydrocarbon chain and a determined number of oxygen groups (hydroxyl, acetoxyls, ketones, epoxy). Most of the acetogenins found in *A.muricata* contain a THF ring, although acetogenins have also been reported with two adjacent or nonadjacent THF rings. Acetogenins are linear and may have one or two epoxy groups.

Cancer is the leading cause of death in the developed world and the second-leading cause of death in developing countries. As of 2012, more than 60% of the cancer cases come from the developing countries. That number represents two-thirds of the deaths caused by cancer, which are 8.1 million people. Treatment of cancer using conventional chemotherapy is quite expensive and has negative side effects, such as the decreased function of the healthy organs. Therefore, treatment using bioactive compounds obtained from plants would be a promising alternative approach. The lactone ring present in acetogenin molecules plays an important role in the anticancer mechanism present[4]. Annonacin is known as the most prevalent acetogenin present in soursop leaves (Champy et al., 2004 and Yuan et al., 2003), however, the method for separation of annonacin from soursop leaves is not widely reported. More reports are available on various methods to obtain new acetogenins from soursop leaves (Kim et al., 1998 and Zeng et al., 1995). The objective of this research work is to identify and isolate acetogenin from *Annona muricata* by thin layer chromatography and column chromatography by usig kedde's reagent. All these preliminary reports form a primary platform for further pharmacological studies of *Annona muricata*.

MATERIAL AND METHODS

Sample collection and authentication

A.muricata L. Leaves were collected locally at Gudalur, Ooty in the month of July 2016. Leaves were authenticated (BSI/SRC/5/23/2016/Tech/1223) by the authority of the botanical survey of India (BSI), Tamil Nadu Agricultural University, Coimbatore.

Sample preparation

Annona muricata leaves were dried and powdered using mechanical blender and 50g *Annona muricata* powder was macerated with 95% ethanol for 5 days. Using a rotary evaporator ethanol was evaporated and the sludge was redissolved in acetone. The solution was filterated by using a Buchner funnel with silica gel 60 on a filter paper. F1, F2 and F3 fractions were obtained by using the solvents water, water-ethanol (7:3 v/v), and water-ethanol (1:1 v/v) to leach the solid crude extract. Then ethanol, ethanol-ethyl acetate (1:1 v/v), and ethyl acetate were used consecutively, combined and evaporated using a rotary evaporator to obtain fraction F4 (Luna et al., 2006).

Identification of acetogenin by using kedde reagent

Analytical TLC was carried out on silica gel 60 as stationary phase and chloroform-methanol (9:1) as mobile phase. Acetogenin visualized by spraying kedde reagent on TLC plate. Kedde's reagent which consists of equal volumes of 2% (w/v) solution of 3,5-dinitrobenzoic acid in ethanol and 5.7% (w/v) solution of KOH in ethanol. It forms pinkish purple color spot in the presence of acetogenin.

Isolation of acetogenin by Column chromatography

30cm column was used to fractionate the sample. F4 fraction was sent to an open column chromatography with silica gel 60 as the stationary phase. Solvents used as elutents were hexane, hexane-chloroform (8:2 v/v), hexane-chloroform (1:1 v/v) and ethyl acetate. The positive fraction was determined by addition of kedde reagent. And the positive fraction was rechromatographed on TLC sheet with slica gel 60 as stationary phase and chloroform-methanol (9:1 v/v) as stationary phase for further confirmation. Pink colour spot on TLC sheet after dipping in kedde reagent confirm the presence of acetogenin in CC fraction.

RESULTS AND DISCUSSION

Identification of acetogenin by using kedde test

All the fractions (F1, F2, F3 and F4) were carried out on analytical TLC sheet with silica gel 60 as stationary phase and chloroform-methanol (9:1) as mobile phase. F4 fraction showed the formation of Pinkish purple color spot on TLC sheet after dipping in kedde's reagent which indicates the presence of acetogenins (shown in Fig 1) and this spot will disappears within a minute. There is no formation of pink spot on all other fractions (F1, F2 and F3) which indicates the absence of acetogenins.

Isolation of acetogenin by Column chromatography

Positive fraction F4 was sent to an open column chromatography with silica gel 60 as the stationary phase. Hexane, hexane-chloroform (8:2 v/v), hexane-chloroform (1:1 v/v) and ethyl acetate solvents were used as eluents. Ethyl acetate fraction showed the positive reaction with kedde's reagent which indicated by the formation of pink to reddish color (shown in Fig 2). Other fraction of solvents will not produce pink to reddish color formation after addition of kedde's reagent and the color disappears within a minute. This indicates that ethyl acetate fraction alone contain acetogenin compound. It further confirmed by rechromatographed on TLC with silca gel 60 as stationary phase and chloroform-methanol (9:1) as mobile phase.

Table 1: Column chromatography fractions

Sample	Eluent	Color	Kedde test
F4	Hexane	Green	-
	Hexane-chloroform (8:2)	Green	-
	Hexane-chloroform (1:1)	Yellowish green	-
	Ethyl acetate	light green	+

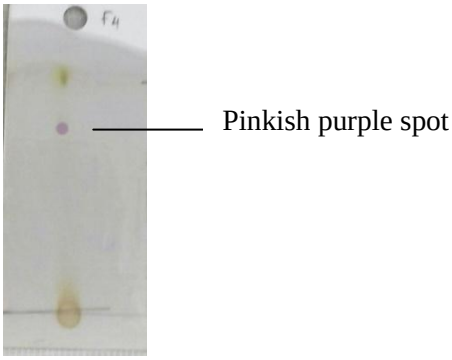

Fig 1: TLC sheet

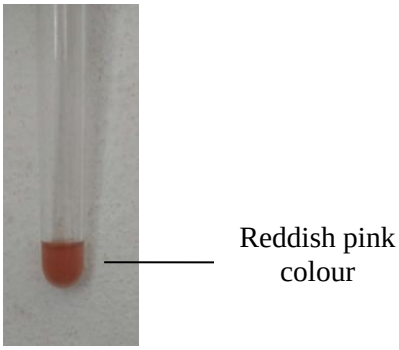

Fig 2: Ethyl acetate fraction (F4)

CONCLUSION

Acetogenins in the *Annona muricata* leaves were fractionated by using open column chromatography and identified by using kedde's reagent which gives the determination of the presence of acetogenin in *Annona muricata*. The presence of acetogenin in the ethyl acetate fraction had an important role in high anticancerous, antitumorous, and antiviral properties. This study shows that acetogenin from *Annona muricata* leaves could be fractionated and enriched using the present open column chromatography and this important phytochemical compound was easily identified by using kedde's reagent.

ACKNOWLEDGMENT

The authors are thankful to the Management, Hindusthan College of Arts and Science, for all the support and encouragement.

REFERENCES

Adewole SO, Caxton-Martins EA. (2006). Morphological changes and hypoglycemic effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on pancreatic B-cells of streptozotocin-treated diabetic rats. Afr. J Biomed Res. 9:173-187.

Champy, P., Hoglinger, G.U., Feger, J., Gleye, C., Hocquemiller, R., Laurens, A., Guerineau, V., Laprevote, O., Medja, F., Lombes, A., Michel, P.P., Lannuzel, A., Hirsch, E.C., Ruberg, M., (2004). Annonacin, a Lipophilic Inhibitor of Mitochondrial Complex I, Induces Nigral and Striatal Neurodegeneration in Rats: Possible Relevance for Atypical Parkinsonism in Guadeloupe. Journal of Neurochemistry, Volume 88, pp. 63-69.

Kim, G.S., Zeng, L., Alali, F., Rogers, L.L., Wu, F.E., Sastrodihardjo, S., McLaughlin, J.L., (1998). Muricoreacin and Murihexocin C, Mono-tetrahydrofuran acetogenins, from the Leaves of Annona muricata. Phytochemistry, Volume 49(2), pp. 565-571

Kreuger, M.R.O., Grootjans, S., Biavatti, M.W., Vandenabeele, P., Herde, K.D., (2012). Sesquiterpene Lactones as Drugs with Multiple Targets in Cancer Treatment: Focus on Parthenolide. Anticancer Drugs, Volume 23(9), pp. 883-896

Luna, J.D.S., Carvalho, J.M.D., Lima, M.R.F.D, Bieber, L.W., Bento, E.D.S., Franck, X., Santana, A.E.G., (2006). Acetogenins in Annona muricata L. (annonaceae) Leaves are Potent Molluscicides. Natural Product Research, Volume 20(3), pp. 253-257

Mishra S, Ahmad S, Kumar N, Sharma BK. (2013). *Annona muricata* (the cancer killer): A review. Glob. J Pharm Res. 2:1613-1618.

Padma, P (2001). "Effect of *Annona muricata* and *Polyalthia cerasoides* on brain neurotransmitters and enzyme monoamine oxidase following cold immobilization stress." J. Natural Remedies 1(2): 144–46.

Yuan, S.S.F., Chang, H.L., Chen, H.W. Yeh, Y.T., Kao, Y.H., (2003). Annonacin, a Mono-tetrahydrofuran Acetogenin, Arrests Cancer Cells at the G1 Phase and Causes Cytotoxicity in a Bax- and Caspase-3-related Pathway. Life Sciences, Volume 72(25), pp. 2853-2861

Zeng, L., (1996). "Five new monotetrahydrofuran ring acetogenins from the leaves of *Annona muricata.*" J. Nat. Prod. 59(11): 1035–42.

Zeng, L., Wu, F.E., McLaughlin, J.L., (1995). Annohexocin, a Novel Mono-THF Acetogenin with Six Hydroxyls, from Annona muricata (Annonaceae). Bioorganic & Medicinal Chemistry Letters, Volume 5(16), pp. 1865-1868

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com