Journal of Medical Pharmaceutical And Allied Sciences
RESEARCH ARTICLE

www.jmpas.com
ISSN 2320-7418

ANTICANCER ACTIVITY OF THE CRUDE EXTRACTS AND AN ISOLATE (β-SITOSTEROL) FROM THE LEAF OF *ANNONA MURICATA*

Nwaehujor Idorenyin Ugochi[1*], Olatunji Gabriel Ademola[2], Das Achana Moni[3]

1. Biochemistry/Chemistry Department, Nigerian Stored Products Research Institute, P.M.B. 1489, Ilorin, Kwara State, Nigeria.
2. Chemistry department, University of Ilorin, Ilorin, Kwara State, Nigeria.
3. Natural product Chemistry Department, North-East Institute of Science and Technology, Jorhat, Assam, India.

Correspondence
Nwaehujor Idorenyin Ugochi
Biochemistry and Chemistry Department, Nigerian Stored Products Research Institute, P.M.B. 1489, Ilorin, Kwara State, Nigeria.
idorenyinugochi@gmail.com

Keywords
Anticancer, Annona, muricata, cytotoxicity, β- sitosterol

Received
25/11/2018
Reviewed
28/11/2018
Accepted
01/12/2018

ABSTRACT

Surgical treatment, radiation therapy and chemotherapy which have been used for the treatment of cancers generally ends up with adverse side effects due to toxicity on human cells. The use of Natural healthy alternatives is now encouraged. Current study was to conduct *in-vitro* anticancer Studies of the ethanol extract and ethyl acetate extract of *Annona muricata* and β-sitosterol isolated from the ethanol extract. *Annona muricata* leaf was dried at ambient temperature and extracted using cold extraction method with increasing polarity of solvents. The solvents used were hexane, ethyl acetate and ethanol. Isolation of β-sitosterol from ethanol extract was carried out using column chromatography. The cytotoxicity of ethanol extract, ethyl acetate extract and β-sitosterol was evaluated *in-vitro* on human cervical cancer (*hela*) cell line. All the samples tested showed different level of cytotoxicity on human cervical cancer cell line. Ethyl acetate extract had highest cytotoxic effect (LC50 = 0.058 µg/mL). The LC50 of ethanol extract was 0.14 µg/mL. The activities of the extracts were higher than the reference standards; doxorubicine (LC50 = 1.725 µg/mL) and docetaxal (LC50 = 28.40 µg/mL). B-sitosterol showed low cytotoxicity (LC50 = 203.56 µg/mL) on *hela* cell line. *Annona muricata* leaf extracts is a promising anticancer agent.

INTRODUCTION

Cancer is the third notable cause of death globally alongside cardiovascular and infectious diseases. Approximately, 12.5 % of the populace dies on account of cancer. [1] The International Agency for Research on cancer evaluation of the prevalence of mortality and prevalence of cancer for 184 countries of the world revealed that there were 14.1 million fresh cancer cases, 8.2 million cancer deaths and 32.6 million persons living with cancer in 2012 worldwide. [2] Cancers are of different types and they are named according to their tissue of origin. Examples, prostate cancer, breast cancer, lung cancer and colon cancer.

It could be caused by human genetics, ionizing radiations, carcinogenic chemical exposure and some pathogens. Treatment protocol varies according to type and stage of cancer. Most treatment includes surgery, chemotherapy and radiotherapy. [3] Even though these methods have been found to be effective in cancer treatment, they are generally connected with serious side effects. For example, fast developing cells are the focus of chemotherapy, so the skin, hair follicles and nail matrix are most often disturbed by chemotherapy [4].

Radiotherapy is also associated with side effects such as skin reactions, hair loss, severe pain, tiredness and lymphodeama. [5] In recent times, medicinal plants play important role in drug discovery and development because of the vast diversity of compounds in plant. [6] The search for anticancer drugs from natural origin began in the late 1960s with the application of podophyllotoxin and its derivatives from the Plant *Polophyllum peltatum*. Progress in the clinical findings for anticancer agents has multiplied over time and new drugs have been discovered. [7]

It has been reported that the use of medicinal plant in the treatment of cancer is advantageous over synthetic drug because adverse side effects are minimized. *Annona muricata* is of the family annonaceae, it is called sour soap because the fruit of the plant has a characteristic sour taste and flavour. [8] *Annona muricata* is native to tropical South and North America, it is now expansively dispersed all over the tropical regions of the world. [9] This study was to investigate the anticancer potential of the ethanol extract, ethyl acetate extract and β-sitosterol isolated from the ethanol extract of *Annona muricata*.

MATERIALS AND METHODS

Preparation and extraction of plant material

The leaves of *Annona muricata* were obtained from a local garden in Ilorin, Kwara State, Nigeria. The sample was identified and documented at the Herbarium of Plant Biology Department, University of Ilorin, Ilorin, Nigeria, with voucher number UIH001/1106. The leaves of *Annona muricata* were dried at ambient temperature, ground to powder using a mill and extracted Using cold extraction method. 2.5 Kg of the Leaf powder was soaked in n-hexane for three days.

The crude extract solution was decanted, filtered and concentrated *in* vacuum. The n-hexane crude extract (52 g; 2.08 %) was coded AMH and preserved in the refrigerator until further analyses. Ethyl acetate was used to soak the remaining plant material for three days. The crude extract was decanted, filtered and concentrated. The ethyl acetate extract was coded AMEA and weighed 110 g; 4.4 %. It was stored in refrigerator until further analyses. The remaining plant material was again soaked in ethanol for three days. The resulting ethanol crude extract which weighed 66 g (2.4 %) was coded AME and preserved in the refrigerator until further Analyses. The crude extracts; n-hexane, ethyl acetate and ethanol were dark green oils.

Isolation and characterization of β-sitosterol by column chromatography

B-sitosterol was isolated from ethanol extract by column chromatography. 10 g of ethanol crude extract (AME) was fractionated by column chromatography with silica gel 230 – 400 mesh size. The column was eluted with n-hexane, ethyl acetate, methanol and ethanol in an increasing order of polarity. Ten fractions were obtained from the column. B-sitosterol was isolated from fraction six using n-hexane and ethyl acetate (49:1) as eluting solvents. The isolate was subjected to various spectroscopic analyses to establish its structure. 1H and 13C NMR were utilized to determine the nature of the protons and carbons present. FTIR was used to determine the functional groups and GC-MS was used to predict the mass of the compound.

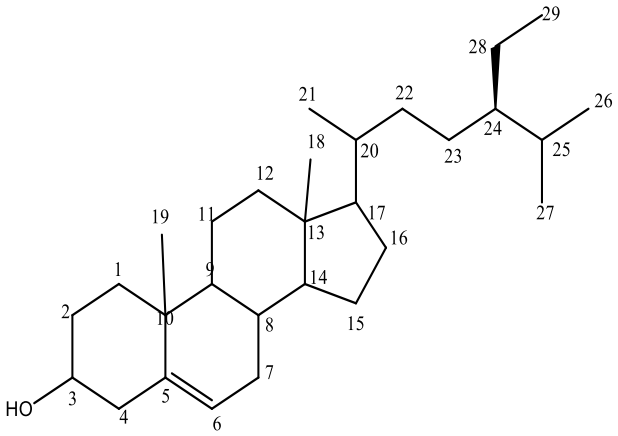
Anticancer assay of the crude extracts and β-sitosterol

Anticancer studies were conducted on ethanol crude extract, ethyl acetate crude extract and β-sitosterol. The cytotoxicity was assessed by *in vitro* assay as described by Senthilraja and Kathiresan, [10] on human cervical cancer cell line (*HeLa* cell line)

procured from National centre for cell science (NCCS), Pune, India. The LC50 of the samples were calculated by analysis of dose response curve.

RESULTS AND DISCUSSION

Characterization of β-sitosterol



The compound was isolated as a white powder (67 mg) from the ethanol extract. The molecular weight is 414 g/mol with formula C29H50O.

IR (CHCl3) Vmax**:** 3429, 2959, 2936, 1666, 1464, 1380, 1050, 772

1H-NMR 500MHz, CDCl3 (ppm)**:** 0.70 (3H, s, H-18), 0.86 (3H, t, J= 6.5Hz, H-29), 0.93 (3H, d, J=5.0Hz, H-26), 0.98 (3H, d, J= 6.5Hz, h-21), 1.03 (3H, s, H-19), 3.53 (1H, m, H-3), 5.36 (1H, d, J= 5.0Hz, H-6)

13C-NMR 500MHz, CDCl3 (ppm)**:** 11.8 (C-29), 11.9 (C-18), 18.6 (C-19), 19.3 (C-21), 19.3 (C-26), 19.7 (C-27), 21.0 (C-11), 22.9 (C-28), 24.2 (C-15), 25.9 (C-23), 28.1 (C- 16) 29.6 (C-27), 31.5 (C-8), 31.8 (C-2), 33.8 (C-22), 36.6 (C-20), 31.5 (C-8), 31.8 (C-2), 33.8 (C-22), 36.6 (C-20), 36.4 (C-10), 37.1 (C-1), 39.7 (C-12), 42.2 (C-13), 45.7 (C-4), 45.7 (C-24), 50.0 (C-9), 55.9 (C-17), 56.6 (C-14), 71.7 (C-3), 121.6 (C-6), 140.6 (C-5)

GC-MS: m/z 414.35

The data is consistent with the reports in literature. [11-13]

Results of anticancer studies

Ethanol crude extract (AME), ethyl acetate crude extract (AMEA) and β-sitosterol were evaluated for their *in-vitro* anti-cancer potential on *HeLa* cell line. AMEA has the highest cytotoxicity (LC50 = 0.058 µg/mL) compared with AME (LC50 = 0.149 µg/mL), AME-6 (LC50 = 203.56 µg/mL) and the reference standards doxorubicine (LC50 = 1.725 µg/mL) and docetaxal (LC50 = 28.40 µg/mL). The effect of the samples on cell viability are shown on fig. 1, Fig. 2 and Fig. 3. The crude extracts AMEA and AME have Higher cytotoxicity index than the reference standards. Their higher cytotoxic index could be attributed to synergetic effects of the various compounds presence in the extracts. Different parts of *A. muricata* have been assayed for cytotoxicity on different cancer cells. [14], reported that aqueous extract of *A. muricata* leaf was cytotoxic on breast cancer cell line. [15], carried out *in-vitro* anticancer

studies on the methanol extracts of the leaves and bark of *A. muricata* using breast cancer cell line. They reported that methanol leaf extract has outstanding anticancer activity in human cancerous cell and the bark extract was not significantly active. From the activity of AMEA and AME on *Hela* cell line used for this study, *A. muricata* leaf can be utilized for the treatment of cancer.

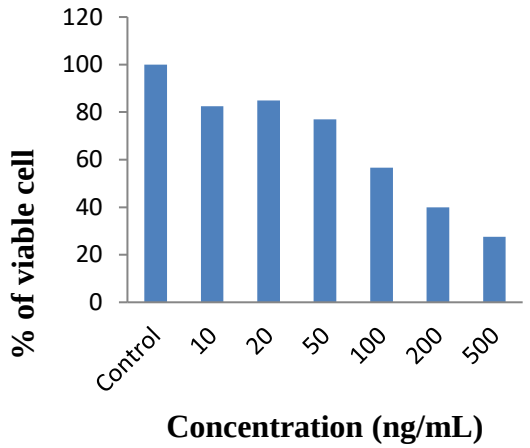


Fig. 1: Effect of ethanol extract on cell viability

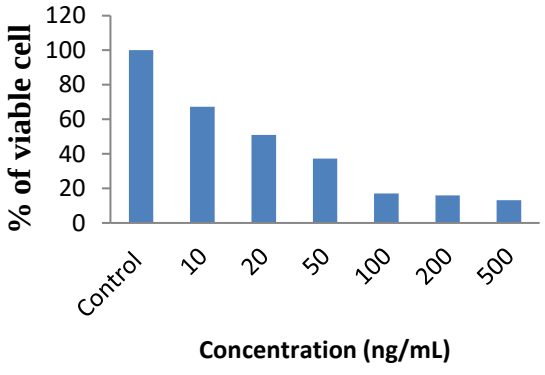


Fig. 2: Effect of ethyl acetate extract on cell viability

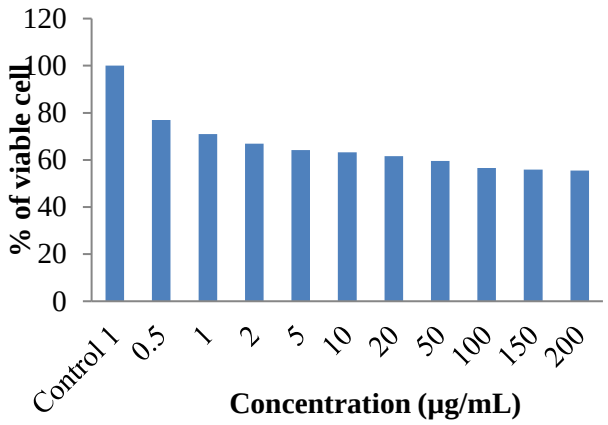


Fig. 3: Effect of β-sitosterol on cell viability

CONCLUSION

Annona muricata leaf can be considered as a source for the isolation and development of novel and effective anticancer agents considering its activity on the human cervical cancer cell lines.

ACKNOWLEDGEMENT

The authors appreciate the Director of North-East Institute of Science and Technology (NEIST), Dr. D. Ramaiah, for providing necessary facilities for this research.

FUNDING

The authors are grateful to The World Academy of Science (TWAS), Trieste, Italy and Council of Scientific and Industrial Research (CSIR), India for sponsoring this research.

REFERENCES

1. Shaikh R, Pund M, Dawane A, Iliyas S, 2014. *J Tradit Complement Med*. 4(4): 253-257.
2. Solowey E, Lichtenstein M, Sallon S,Paavilainen H, Solowey E, Lorberboum-Gakski H, 2014. Evaluating medicinal plants for anticancer activity. *Scienc world J*. 2014, 1-12.
3. Sheikh R, Shahzad A, Mahwash F, Sadaf D, 2018. Toxicity of anticancer drugs and its prevention with special reference to the role of garlic constituents. *Annals Phytomed*. 7(1): 13-26.
4. Kamil N, Kamil S, Ahmed SP, Ashraf R, Khurram M, Ali MO, 2010. Toxic effects of multiple anticancer drugs on skin. *Pak J Pharm Sci*. 23(1): 7-14.
5. Zhang J, Wel-Tan H, Hu C, Wang W, Chu G, Wei L, Chen L, 2018. Anticancer activity of 23, 24-dihydrocucurbitacin B against the *Hela* human cervical cell line is due to apoptosis and G_2/M cell cycle arrest. *Therap med*. 15: 2575-2582.
6. Lowe H, Toyang NJ, Watson C, Bodal S, Bahado-Singh P, Bryant J, 2013. *In-vitro* anticancer activity of the crude extract and two dicinnamate isolates from the Jamaican ball moss (*Tillandsia recurvata L.*). *Am Int J Contemp Res*. 3(1): 93-96.
7. Narah M, Kalita JC, Kotoky J, 2012. Medicinal plants with potential anticancer activities: A review. *Int Res J Pharm*. 3(6): 26-30.
8. Bora PS, Holschuh HJ, Vasconcelos MA, 2004. Characterization of polyphenol oxidase of soursop (*Annona muricata L.*) fruits and a comparative study of its inhibition in enzyme extract and pulp. *Cienc Tech Ali* 4(4): 267–273.
9. Moghadamtousi SZ, Rouhollahi E, Hajrezaie M, Karimian H, Abdulla MA, Kadir HA, 2015. *Annona muricata* leaves accelerate wound healing in rats via involvement of Hsp70 and antioxidant defence. *Int J Surg.* 18: 110–117.
10. Senthilraja P, Kathiresan K, 2015. *In-vitro* cytotoxicity MTT assay in Vero, Hep G2 and MCF-7 cell lines study of marine yeast. *J App Pharm Sci*. 5(3): 80-84.
11. Lee K, Kim MA, Lee SY, Hong JK, Lee JH, Lee KR, 2008. Phytochemical constituents of *Schizonepeta tenuifolia* Briquet. *Nat Prod Sci*. 14(2): 100–106.
12. Rajput AP, Rajput TA, 2012. Isolation of stigmasterol and β-sitosterol from chloroform extract of leaves of *Corchorus fascicularis* Lam. *Int J bio Chem*. **6**: 130–135.

13. Saeidnia S, Manaji A, Gohari AR, Abdollahi M, 2014. The story of β-sitosterol – A Review. *Euro J Med Plants*. 4(5): 590–609.
14. Najmuddin SU, Romli MF, Hamid M, Alitheen NB, Abd Rahman NM, 2016. Anti-cancer effect of *Annona muricata* Linn leaves crude extract (AMCE) on breast cancer cell line. BMC *Complement Alt Med*. 16: 311–331.
15. Bhaumik Thakar*, Dr. Alka Agarwal, Dr. Rakesh M Rawa, 2017. Indian food and spices as a Cancer remedies. Journal of Medical Pharmaceutical and Allied Sciences (Vol-6_I-11_November) 03; 904-917
16. Arun R, Philip A, Kannan P, John N, 2014. Screening of anticancer and antibacterial activity of methanolic activity of *Annona muricata* leaf and bark. *Innor Int J Sci.* 2(3): 1–4.