



Research article

Anticancer activity of the crude extracts and an isolate (β -SITOSTEROL) from the leaf of *Annona muricata*

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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 ($LC_{50} = 0.058 \mu\text{g/mL}$). The LC_{50} of ethanol extract was $0.14 \mu\text{g/mL}$. The activities of the extracts were higher than the reference standards; doxorubicine ($LC_{50} = 1.725 \mu\text{g/mL}$) and docetaxal ($LC_{50} = 28.40 \mu\text{g/mL}$). B-sitosterol showed low cytotoxicity ($LC_{50} = 203.56 \mu\text{g/mL}$) on hela cell line. *Annona muricata* leaf extracts is a promising anticancer agent.

Keywords: Anticancer, *Annona muricata*, Cytotoxicity, β - sitosterol.

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. 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. 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. 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.

Radiotherapy is also associated with side effects such as skin reactions, hair loss, severe pain, tiredness and lymphodeama. In recent times, medicinal plants play important role in drug discovery and development because of the vast diversity of compounds in plant. The search for anticancer drugs from natural origin began in the late 1960s with the application of podophyllotoxin and its derivatives from the Plant *Podophyllum peltatum*. Progress in the clinical findings for anticancer agents has multiplied over time and new drugs have been discovered. 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. *Annona muricata* is native to tropical South and North America, it is now expansively dispersed all over the tropical regions of the world. 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* [1, 2].

METHODOLOGY

Preparation and extraction of plant material. The leave 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 leave 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 β -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. β -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 ^{13}C 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

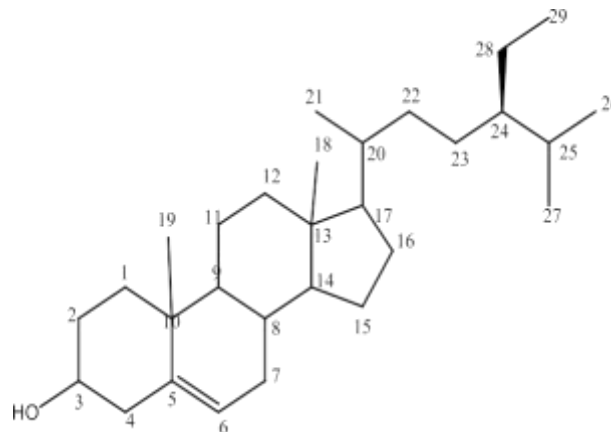
Anticancer studies were conducted on ethanol crude extract, ethyl acetate crude extract and β -sitosterol. The cytotoxicity was assessed by in vitro assay as described by Senthilraja and Kathiresan, on human cervical cancer cell line (HeLa cell line) procured from National centre for cell science (NCCS), Pune, India. The LC₅₀ of the

samples were calculated by analysis of dose response curve [3-7].

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) V_{max}: 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)

^{13}C -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.

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 $\mu\text{g/mL}$) compared with AME (LC₅₀ = 0.149 $\mu\text{g/mL}$), AME-6 (LC₅₀ = 203.56 $\mu\text{g/mL}$) and the reference standards doxorubicine (LC₅₀ = 1.725 $\mu\text{g/mL}$) and docetaxal (LC₅₀ = 28.40 $\mu\text{g/mL}$). 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., reported that aqueous extract of *A. muricata* leaf was cytotoxic on breast cancer cell line., 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 [8-12].

Figure 1: Effect of ethanol extract on cell viability

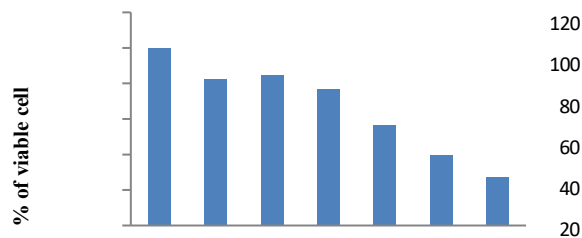


Figure 2: Effect of ethyl acetate extract on cell viability

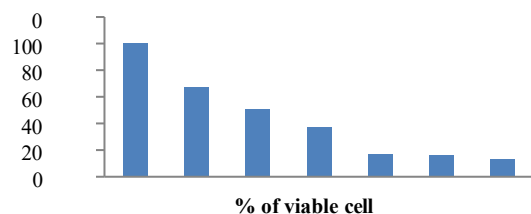
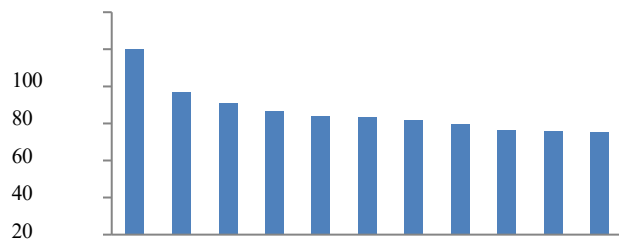


Figure 3: Effect of β -sitosterol on cell viability



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.

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REFERENCES

1. Solowey E, Lichtenstein M, Sallon S, 2014. Evaluating medicinal plants for anticancer activity. Scienc world J. Pages 1-12. Doi: 10.1155/2014/721402.
2. Sheikh R, Shahzad A, Mahwash F, 2018. Toxicity of anticancer drugs and its prevention with special reference to the role of garlic constituents. Annals Phytomed. 7(1), Pages 13-26. Doi: 10.21276/ap.2018.7.1.3.
3. Zhang J, Wel-Tan H, Hu C, 2018. Anticancer activity of 23, 24-dihydrocucurbitacin B against the Hela human cervical cell line is due to apoptosis and G2/M cell cycle arrest. Therap med. 15, Pages 2575-2582. Doi: 10.3892/etm.2018.5710.
4. Lowe H, Toyang NJ, Watson C, 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), Pages 93-96.

5. Narah M, Kalita JC, Kotoky J, 2012. Medicinal plants with potential anticancer activities: A review. Int Res J Pharm. 3(6): 26-30. Doi: <https://doi.org/10.55230/mabjournal.v52i1.2274>.
6. 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), Pages 267–273. Doi: 10.1080/11358120409487770.
7. Akio M, Tomoki N, Tatsuo W, 1988. Pattern differences in experimental fevers induced by endotoxin endogenous pyrogens and prostaglandins. Am J Physiol. 254, Pages 633-640. Doi: 10.1152/ajpregu.1988.254.4.R633.
8. Singh o, 2012. Phytochemical investigation and antifungal activity of the seeds of *Centrathrum anthelminticum*; Kuntze, Acta Poloniae Pharmaceutical Drug Research. 69(6), Pages 1183-1187. Doi: 10.3923/ijp.2013.211.226.
9. Lateef M. Khan, Sameer E. Al-Harhi, Omar I. Saadah 2013. Adverse drug reactions in hospitalized pediatric patients of Saudi Arabian University Hospital and impact of pharmacovigilance in reporting ADR. Saudi Pharmaceutical Journal. 21, Pages 261–266. Doi: 10.1016/j.jsps.2012.09.004.
10. Padmaja Uday Kumar, Prabha Adhikari, Pratibha Periera, 2009. A Prospective Analysis of Adverse Drug Reactions in a South Indian Hospital. Online Journal of Health and Allied Sciences. 8(3), Pages 1-6. Doi: <http://www.ojhas.org/issue31/2009-3-12.htm>.
11. Joel Thomas, Joshin Sara Cherian, Feba Susan Varghese, Shelina Hephzibah S, Vijay MC, Lijo James, 2018. Clinical Adverse Drug Reaction of Anti-Neoplastic Agents. JMPAS, jour. Of med. Phar and allied sci. V 7 - I 2, 755, 2018, 1000-1007.
12. 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), Pages 80-84. Doi: 10.7324/JAPS.2015.50313.

% of viable cell