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Cytotoxic Assessment of *Xylopia aethiopica* [Dun.] A. on Human Prostate and Breast Cancer Cell Lines

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ABSTRACT

Cancer is associated with the unregulated multiplication of cells in specific parts of the body. Plant-derived products are employed in the treatment and management of such malignant tumours. Prostate and breast cancers are the most common forms affecting Nigerians. Our aim is to investigate the cytotoxic effect of *Xylopia aethiopica*, a commonly consumed spice in Nigeria, on selected cancer cell lines. The antiproliferative activity of *X. aethiopica* ethanol extract of varying concentrations was assessed on MDA-MB-231, MCF-7, and LNCaP using MTT assay. A dose-dependent antiproliferative activity was observed. The IC_{50} for *X. aethiopica* on the cell lines were ≤ 4.506 ± 0.07 µg/mL except for MCF-7 at 72 hours, where 18.86 ± 0.20 µg/mL was recorded. The findings from this study indicate the potential antiproliferative and cytotoxic property of *X. aethiopica* on human prostate and breast cancer cell lines.

Keywords: *Xylopia aethiopica*, Cell viability, MDA-MB-231, LNCaP, Medicinal plants, Cancer.

Introduction

The World Health Organization (WHO) 2018 report placed cancer as the second leading cause of death globally. Of the different cancer forms, breast cancer accounts for the second-highest incidence (2.09 million cases) and prostate cancer, the fourth (1.28 million cases) in 2018. In developing countries, approximately 70% of deaths are due to cancer.[1] Cancer Research UK predicts that there will be 27.5 million new cases each year of cancer globally by 2040 if the current trend persists.[2] The burden of prostate and breast cancer are under-reported in Nigeria. Nigeria's cancer country profile 2020 shows that Nigeria recorded an estimated 115 950 cancer cases and 70 327 deaths from all cancers in 2018. A large proportion of the population never seeks orthodox medical care. As a result, such cases are not recorded. Breast cancer develops from abnormal proliferation of breast tissues. The female hormones (estrogen and progesterone) have been reported to promote the growth of breast cancer.[3] inversion of the nipple, nipple discharge, abnormal thickening or lump in the breast, and persistent pain in parts of the breast are some symptoms associated with breast cancer.[4] The underlying cause is unknown, but potential risk factors include age, sex, race, genetics, lifestyle (e.g., nulliparity, lack of breastfeeding), use of hormonal contraceptives or replacement therapy, diet, and obesity.[5] Screening for breast cancer can be carried out through physical examination of the breasts, mammography, or biopsy.[6] The outcome of a diagnosis of breast cancer is dependent on the age of the patient and the stage of cancer. Some treatment options include; surgery, chemotherapy, radiation therapy, immunotherapy, and hormone therapy. Prostate cancer, on the other hand, occurs in the male organ called the prostate. It may present with difficulty, pain during urinating, and erectile dysfunction. The underlying cause of prostate cancer is unknown, but some of the risk factors include age, diet, alcohol consumption, occupation, race, lifestyle, etc. Active surveillance of prostate cancers involves regular screening with a prostate-specific antigen (PSA) blood test and a digital rectal exam (DRE).[7] Treatment and management options for prostate cancer include hormonal therapy, chemotherapy, cryosurgery, and prostatectomy. The adoption of plants in treating ailments and diseases has long been a practice since ancient times. As a result, most traditional anticancer drugs are of plant origin or derivatives of plants.[8] According to Iweala *et al*.,[9] WHO had reported that greater than eighty percent of the populace in low- and middle-income countries rely on herbal formulations. Asadi-Samani *et al*.[10] reported the positive results obtained from using plants to treat cancer. Medicinal plants employed in managing cancer in southwestern Nigerian and other plants with cytotoxic potentials have been reported in literature.[11-13] Plants are rich in phytochemicals with potential biological effects that can be employed in combating cancer. *Xylopia aethiopica* is a medicinal plant native to Africa, mainly Nigeria, Senegal, and East Africa. It is an aromatic evergreen tree of the family Annonaceae growing up to 20 m in height. Its fruits have an attractive spicy flavour when dried through smoking. It is commonly known as Negro pepper.[14] Some of its ethnomedicinal uses include; treating snakebites, headache, diabetes, stomach ache, dysentery, cardiovascular diseases, wound dressing, cancer, and epilepsy.[15] The phytochemicals present in the fruits of *Xylopia aethiopica* include saponin, saponin glycoside, tannin, and cardiac glycoside.[16] The cytotoxic effect of *X. aethiopica* has been reported for some human cancer cell lines such as leukemia, pancreatic, colorectal, and breast carcinoma (SUM-159PT).[17] This study aims to investigate the potential cytotoxic effect of ethanol extract of *Xylopia aethiopica* on selected human cancer cell lines from the breast (MDA-MB-231, a triple-negative breast cancer cell line, and MCF-7, an estrogen and progesterone receptor-positive but HER2 negative cell line) and prostate (LNCaP) cancer which may further give credence to its use with scientific backing in the management of breast and prostate cancer, two of the most prevalent forms of cancer in Nigeria.

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Materials and Methods

Chemicals and reagents

Androgen-sensitive human prostate adenocarcinoma cells (Lymph Node Carcinoma of the Prostate, LNCaP), Human breast adenocarcinoma (MCF-7), and Metastatic human breast adenocarcinoma (MDA-MB-231) were obtained from ATCC, Rockville, MD, USA. Dulbecco's modified eagle medium (DMEM) was gotten from Life Technologies GIBCO, Grand Island, NY, USA. Ethanol, fetal bovine serum (FBS), and all other reagents used were of analytical grade and acquired from Sigma-Aldrich St. Louis, MO.

Collection of plant material

Dried fruits of *X. aethiopica* were purchased from a market in Ota, Ogun State, Nigeria, and were identified by a taxonomist Dr. J. O. Popoola of the Department of Biological Sciences, Covenant University, Ota, Nigeria. The voucher specimen of *X. aethiopica* (CUBIO0211 - *Xylopia aethiopica* (Dunal) A. Rich. (Annonaceae) was deposited at the herbarium of the Department of Biological Sciences, Covenant University, Ota, Ogun State. The dried fruits were finely ground using a commercial grinder (M6FFC-160 grain milling machine, ABC Machinery Engineering Co., Ltd, China) and stored in an airtight container.

Preparation of plant extract

The extract was obtained by soaking the ground fruit (400 g) for 2 h (shaking at intervals of 30 min) with 70% ethanol (100 mg/mL), followed by centrifugation of suspension for 10 min at 4000 rpm and subsequent filtration and storage of supernatant at -20^{o}C.

Cell line maintenance

The cancer cell lines were maintained in DMEM supplemented with 10% FBS, glutamine, phenol red, and 50 µg/mL gentamycin in a humidified atmosphere at 37°C and 5% CO_2.[18] Cell counting was done manually, as described by Cadena-Herrera *et al.*[19]

In vitro cytotoxicity and cell viability assay

Cytotoxicity and cell viability were measured using MTT (3-(4, 5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) assay to determine the activity of mitochondrial enzymes through the reduction of MTT to formazan. Cells were briefly seeded onto RPMI1640 culture medium in 96-well at the rate of 2500 cells per well. The cells were washed after twenty-four hours, and 100 µL of cells were placed in culture media containing varying amounts (1, 10, 50, and 100 µg/mL) of extract for 48 h and 72 h. Then, 100 µL of non-FBS culture medium containing 10 % MTT (5 mg/ml) was placed into each well, and the samples were left to stand for four hours at 37^{o}C. The culture medium was discarded, and 100 µL DMSO was added to each well. The absorbance was taken at 540 nm with the aid of an enzyme-labeled detector (Multiscan MK3, Thermo Electron, USA). No extract was administered to the control cells and their absorbance taken as 100%. This assay was done in quadruplicates.

$$\text{Cell viability} = \frac{AbsT - AbsB}{AbsC - AbsB} \times 100$$

Where AbsT = Absorbance of test, AbsB = Absorbance of blank, AbsC = Absorbance of control

The IC_{50} values for LNCaP, MCF-7, and MDA-MB-231 were determined through linear regression with the aid of the GraphPad Prism 6.

Statistical analyses

The statistical package SPSS 16.0 for windows was used in analyzing experimental data. Results were expressed as mean ± SEM. Statistical analysis was carried out by one-way ANOVA supplemented with Duncan *post-hoc* analysis considered statistically significant at $P < 0.05$.

Results and Discussion

At the end of 48 h exposure to varying concentrations of *X. aethiopica* extract, the cell lines showed a significant decrease in percentage cell viability except for LNCaP at 1µg/mL, as shown in Figure 1. The highest reduction in cell viability was observed at 100 µg/mL.

After 72 h, a significant decrease in % cell viability was observed in all cell lines except at 1 µg/mL of extract, as shown in Figure 2. 100 µg/mL of the extract also had the highest inhibitory effect on cell viability.

The extract showed positive antiproliferative potential for the cell lines used due to the low IC_{50} values recorded, as shown in Table 1.

The fight against cancer is an on-going battle. Cases of multidrug resistance, relapse, and severe side effects from chemotherapeutic treatments keep rising. Plants have always been useful resources in researchers' hands for the identification and invention of novel therapeutic drugs as a result of their effective utilization in the traditional treatment of diseases such as cancer.[20] Female breast cancer and prostate cancer are among the forms of cancer with the highest incidence and tend to be prevalent among Africans and people of African ancestry. Africans are known to enjoy spicy foods; thus, employing such spices with anticancer properties in the prevention, management, and treatment of this disease will go a long way in reducing the incidence and mortality rates presently being recorded. Antiproliferative and anti-oxidative effects of *X. aethiopica* on selected cell lines of cancer have been reported previously. This study's result affirmed previous reports of the cytotoxic property of *X. aethiopica* on cancer cell lines.[9, 17, 21] This study is the first to investigate its cytotoxic property on the MDA-MB-231 cell line. Our data showed that *X. aethiopica* fruit extract effectively inhibited the viability of all the cell lines studied in a concentration-dependent manner after 48 h (Figure 1) and 72 h (Figure 2). After 48 h, there was no significant difference in the percentage cell viability of MDA-MB-231 as the concentration of extract administered increased from 10 µg/mL to 100 µg/mL. Simultaneously, there was a significant difference in the percentage cell viability of both MCF-7 and LNCaP at extract concentration of 50 µg/mL and 100 µg/mL (Figure 1). From Fig. 2, MDA-MB-231 and LNCaP had significantly different cell viability at the extract concentration of 10 µg/mL and 50 µg/mL but none at 100 µg/mL, while MCF-7 had a significant decrease in percentage cell viability at 100 µg/mL. The result of this study affirmed previous reports of the cytotoxic property of *X. aethiopica* on cancer cell lines. Overall, *X. aethiopica* inhibited the viability of the LNCaP cell line (prostate cancer) (Figures 1 and 2). This suggests that it may be useful in the management of prostate cancer. Although there are reports of reduction in sperm count and motility from the administration of *X. aethiopica*,[22, 23] Abarikwu *et al*. [24] reported that the compounds with infertility effects were found in the seeds than the pods. Also, most men diagnosed with prostate cancer are usually advanced in age and may not be too keen about procreating. MDA-MB-231 and MCF-7 showed sensitivity to *X. aethiopica* from the results. There has been a report of its cytotoxic effect on MCF-7.[9] The effect observed on MCF-7 cells is similar to the findings of Bakarnga-Via *et al.*[25] which showed that essential oils extracted by hydrodistillation of *Xylopia aethiopica* fruits have cytotoxic effects on MCF-7 breast cancer cell line with a more pronounced effect on neoplastic cells and little or no effect on normal cells. *X. aethiopica*'s cytotoxic effect on the cancer cell lines may be due to its antioxidant potential.[26, 27] Both MDA-MB-231 and LNCaP had significantly different IC_{50} from MCF-7 after 48 h and 72 h, suggesting that though the extract is effective against both the prostate and breast cancer cell lines, there might be variations in its level of cytotoxicity on different breast cancers based on the presence or absence receptors. The IC_{50} for the cell lines was less than or equal to 4.506 µg/mL for 48 h and 72 h, except for MCF-7 at 72 h, which had an IC_{50} of 18.86 µg/mL (Table 1). These values are below the American National Cancer Institute's (NCI) recommended upper IC_{50} limit of 30 µg/mL for purification of crude extracts,[28, 29] validating the use of *X. aethiopica* in the prevention and treatment of cancer. The presence of significant alkaloids, saponin, and flavonoids in *X. aethiopica* [30] also gives credence to its use as an antitumor agent.[31]

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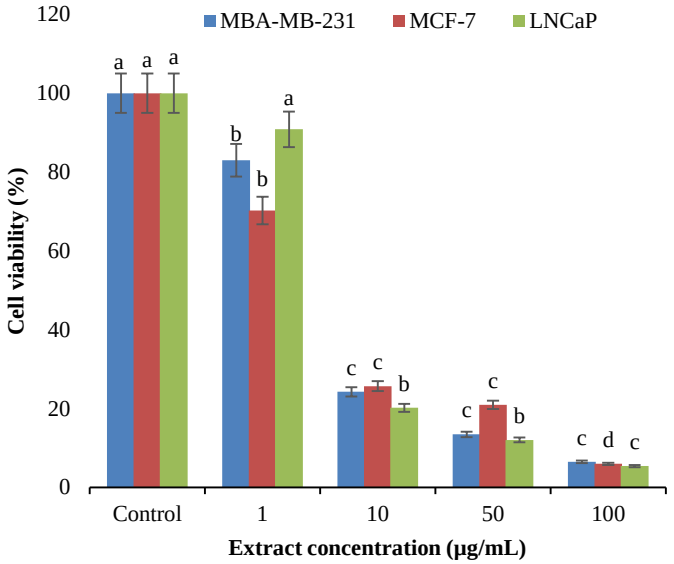

Figure 1: Percentage cell viability in the presence of different concentrations of *X. aethiopica* extract after 48 h.
Bars are expressed as mean ± SEM. Different superscript letters across concentration bars denotes significant difference at $p < 0.05$.

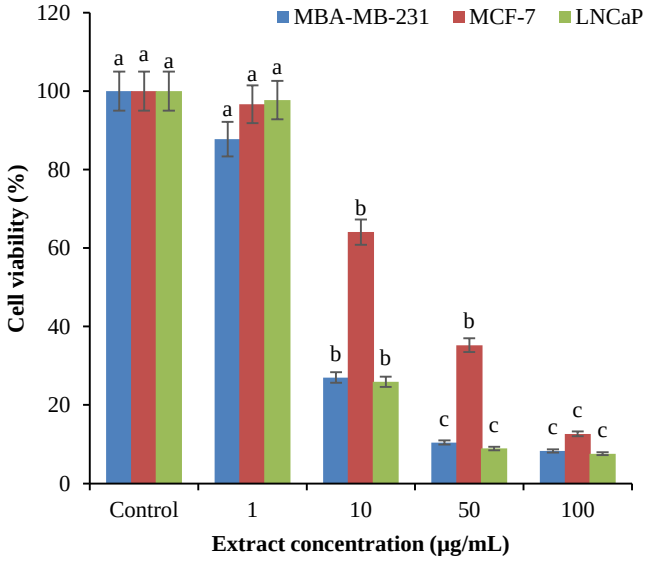

Figure 2: Percentage cell viability in the presence of different concentrations of *X. aethiopica* extract after 72 h.
Bars are expressed as mean± SEM. Different superscript letters across concentration bars denotes significant difference at $p < 0.05$.

Table 1: IC_{50} values of *Xylopia aethiopica* extract

	IC_{50} (µg/mL)		
Time	**MDA-MB-231**	**MCF-7**	**LNCaP**
48h	3.408 ± 0.04^{b}	2.064 ± 0.15^{a}	3.371 ± 0.09^{b}
72h	3.987 ± 0.10^{a}	18.86 ± 0.20^{b}	4.506 ± 0.07^{a}

Results are expressed as mean± SEM. Different superscript across a row denotes significant difference at $p<0.05$. Abbreviation: IC_{50}: Half maximal inhibitory concentration

Conclusion

In conclusion, the ethanol extract of *X. aethiopica* was cytotoxic against all the cell lines studied. For the breast cancer cell lines, it was more effective against MDA-MB-231 than MCF-7, suggesting that it is more effective against receptor-negative subtypes. The IC_{50} value was within the range recommended by the American National Cancer Institute.[28]

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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