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Antiproliferative Activity of Ethanol Extract of *Boswellia dalzielii* (Hutch) Stem Bark in Breast Cancer Cell Line

A. P. Otitoju[1,2,3*], I. Y. Longdet[1], T. E. Alemika[4] and V. P. Gota[3]

[1]Department of Biochemistry, Faculty of Medical Sciences University of Jos, Jos, Nigeria.
[2]Medical Biotechnology Department, National Biotechnology Development Agency, Abuja, Nigeria.
[3]Clinical Pharmacology Department, Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi-Mumbai, 410210, India.
[4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Jos, Jos, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author APO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IYL and TEA managed the analyses of the study. Authors APO and IYL managed the literature searches. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aim and Objectives: The present study aimed to examine the antiproliferative activity of ethanol extract derived from the *Boswellia dalzielii* stem bark in breast carcinoma.
Methodology: Ethanolic extract of stem bark of *Boswellia dalzielii* were prepared. Antiproliferative activity was assessed in estrogen receptor (ER)-negative breast carcinoma (MDA-MB-231) cells by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay.
Results: Obtained results in 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay indicated that ethanol extract of *Boswellia dalzielii* stem bark showed significant antiproliferative activity in MDA-MB-231 cells in a dose-dependent manner. IC50 of Boswellia dalzielii stem bark ethanol extract in MDA-MB231 was 98. 12 µg/mL.

**Corresponding author: E-mail: akinbobolapeace@gmail.com;*

Conclusion: The results obtained suggest that *Boswellia dalzielii* stem bark extract possesses significant antiproliferative potential which could be mediated by the chemical constituents present in the plant. However, further research needs to be carried out to determine the effect of the extract on the cell cycle and also to determine the type of cell death produced by the extract.

Keywords: Antiproliferative activity; MDA-MB231; Boswellia dalzielii.

1. INTRODUCTION

During the last few years, the morbidity and mortality associated with estrogen receptor (ER) positive breast cancer has decreased due to the introduction of drugs such as the aromatase inhibitors and selective estrogen replacement modulators (SERMs) [1], whereas, the more malignant and aggressive estrogen receptor (ER) negative breast cancer is unresponsive to hormonal treatment, and the current therapeutic modalities are associated with toxicity and side effects [2]. In this task, treatments derived from natural products have been considered since they are claimed to be an important source for drug candidates.

Boswellia dalzielii (Ararrabi, Basamu or Hanu in Nigerian local dialect) trees are characteristically about 13m tall, possessing compound leaves, papery bark and star-like flowers [3,4]. It is used in ethnomedicine to treat a host of diseases including gastrointestinal disorders, leprosy, rheumatism and various diseases of microbial origin [5,6]. Some research groups have reported the cytotoxic activity of *Boswellia dalzielii* extracts in brine shrimp [7], ovarian tumour cells OVCAR-3 and IVROV-1 [8], and more recently in breast cancer cell line, MDA-MB231 [9], and oral squamous cell carcinoma, AW8507 cell line [10]. Furthermore, Kafuti et al. [11] earlier reported the antiproliferative properties of *Boswellia dalzielii* stem bark on Sorghurm bicolor. The aqueous (dialyzed) extract of *Boswellia dalzielii* dried resin from Cameroon [12], and the methanol extract and fractions of Boswellia dalzielii stem bark [13], demonstrated anti-inflammatory activity in rats. Boswellia dalzielii has also been reported to have hepatoprotective efficacy. Aliyu et al. [14] investigated the effect of aqueous bark extract of *Boswellia dalzielii* on liver function of female Wistar albino rats, the extract reportedly had no significant effect on total protein, albumin, bilirubin content and alkaline phosphate activity at the end of five days oral treatment. methanolic extracts of *Boswellia dalzielii* leaf were assessed for their influence on the liver of male albino rats, *Boswellia dalzielii* reportedly demonstrated significant hepatoprotection in male wistar rats induced with carbon tetrachloride (CCL4) toxicity [15]. The earlier scientific research reports and the traditional use of *Boswellia dalzielii* has stimulated our study; anti-proliferative effect of ethanol extract of *Boswellia dalzielii* (Hutch) stem bark in MDA-MB-231 using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay.

2. MATERIALS AND METHODS

2.1 Plant Source

Boswellia dalzielii stem bark (BDB) was collected from Dutsen Hanwa area of Zaria, Kaduna state, Nigeria in the month of November. The plant was identified and authenticated at Ahmadu Bello University, Biological Sciences Department. A voucher specimen was deposited with voucher number 900121.

2.2 Extraction

Extraction of the *B. dalzielii* stem bark was as described earlier [12]. Briefly, fresh *Boswellia dalzielii* stem bark (BDB) was washed in water, air dried at room temperature and pulverized. Two kilograms (2.0kg) of powdered material were extracted with 70% ethanol (50g BDB/200ml ethanol) at room temperature and evaporated to yield a dark-brown jelly (180g) which is 9% of the plant's starting material.

2.3 Cell Culture

The estrogen negative (ER) breast cancer cells (MDA-MB231) were generously obtained from the Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Kharghar, Navi-Mumbai, India. Cells were cultured in Dulbecco's Minimum Essential Medium (DMEM) with 10% fetal bovine serum (FBS) and 50µg/mL gentamicin. The cells were incubated at 37°C in CO_2 incubator in an atmosphere of humidified 5% CO_2 and 95% air. The cells were maintained by sub-culturing in 25cm^3 tissue culture flasks. Cells growing in the exponential phase were used for cell viability assay.

2.4 Cell Toxicity Assay

The antiproliferative effect of the crude ethanolic extract of *B. dalzielii against* MDA-MB231 cells was determined according to the method earlier described [10,16]. Briefly, cells were seeded into 96-well microplates at the density of 4 x 10^3 cells/well. After 24 h of culture, the medium was removed and replaced with a fresh medium containing various concentrations (0, 25, 50, 100, 150, 200, and 250µg/mL) of *B. dalzielii* stem bark ethanolic extract or 2.5% ethanol in DMEM as the vehicle control. Following 24 h incubation, the media was discarded, cells were washed with PBS once (thus removing the interference of the polyphenols with MTT assay) and 100µl of MTT solution (0.5 mg/mL in serum and phenol red free DMEM) was added to each well, incubated for 4 h at 37^{o}C. Afterwards, the solution was discarded and 100µl of DMSO was added to each well and incubated in the dark for 1 hour. Thereafter, absorbance was measured at 570 nm. Three independent experiments were carried out. The results were expressed as the mean percentage ± standard deviation (Mean % ± STDEV) of viable cells compared to the control cells. MTT test is based on the reduction of the yellow soluble compound 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich Chemical, Germany) into an insoluble formazan (blue star-shaped crystals). The reaction takes place in the mitochondrial membrane of living cells. Formazan was dissolved by addition of a strong detergent (DMSO) and color was read spectrophotometrically at a wavelength of 570 nm (cytation5 Cell imaging multi-mode Reader, BioTek Instruments Incorporation, Winooski, Vermont 05404-0998 USA). Absorbance of the solution is proportional to the number of living cells.

2.5 Statistical Analyses

The cytotoxic assay was performed in triplicates and repeated at least two times and the data were presented as mean ± SD. Probability values $P < 0.05$ were considered statistically significant. Half maxima inhibitory concentration (IC50) was determined by Quest Graph IC_{50} Calculator [17].

3. RESULTS AND DISCUSSION

The results as summarized in Fig. 1 are presented as percentage MDA-MB231 cell viability and presented as Mean ± standard deviation (n = 3). The results showed a dose-dependent activity towards the MDA-MB231 cell line. The half maxima inhibitory concentration (IC_{50}) value calculated for *Boswellia dalzielii* stem bark ethanol extract in MDA-MB231 cells was 98.152 µg/mL

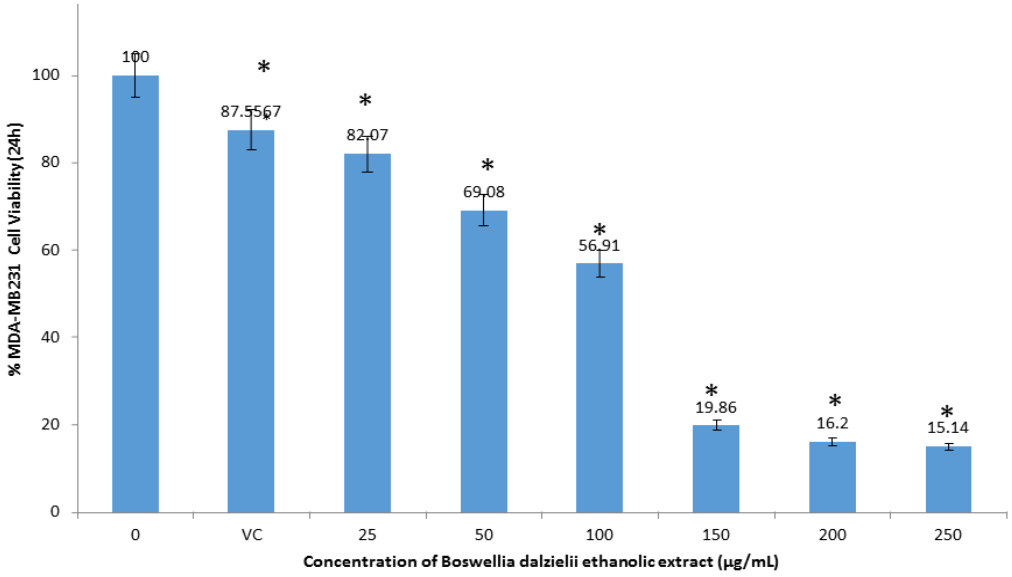

Fig. 1. Cytotoxic effects of ethanolic extract of *Boswellia dalzielii* stem bark on human breast cancer cells

(Mean difference is significant at the 0.01 level). VC contains the percentage of 2.5% ethanol present in the highest plant extract dosage*

Despite important improvements in early detection and treatment, breast cancer continues to be one of the major life-threatening diseases that a woman may face in her life time [18]. Although chemotherapy has become a routine in most anticancer treatments, this therapeutic approach is often limited by the ability of cancer cells to develop resistance to conventional drugs [19]. Therefore, the search for new compounds with selective anticancer activity continues to be an important driving force for the development of novel antitumour agents. The present study aimed at investigating the cytotoxic / antiproliferative activity of the ethanolic extract of *B. dalzielii* in breast cancer cells. Few research groups have evaluated the cytotoxic activity of *Boswellia dalzielii* extracts in various cancer cell lines [8,10]. However, the cytotoxicity of *Boswellia dalzielii* stem bark extract was investigated on the MDA-MB231 cell line in this study. *Boswellia dalzielii* has been shown to be an important medicinal plant used in folkloric medicine to cure various diseases in West Africa [6,20,21]. Consistent with this perception, the results of the present study (Fig. 1) indicate that the ethanol extract of *Boswellia dalzielii* stem bark potently inhibited, in a dose-dependent manner, the proliferation of MDA-MB-231 (ER-negative breast cancer cells). These results further lend credibility to an earlier report [9], in which the extract of *Boswellia dalzielii* gum resin was effective against MDA-MB231 cells. The activity of *Boswellia dalzielii* stem bark extract could be attributed to the phytochemicals that have been reported for the plant, including Acetyl-11-keto-beta boswellic acid, Protocatechuic acid, and Gallic acid [22,23,24,25]. This holds promise for further *in vitro* and *in vivo* studies to examine the mechanism of antiproliferative activity of *Boswellia dalzielii* stem bark in estrogen receptor-negative breast cancers.

4. CONCLUSIONS

Our findings show that the ethanol extract of *Boswellia dalzielii* suppressed, in a dose-dependent manner, the proliferation of the breast cancer cell line, MDA-MB-231. This study provides an important basis for further investigation into the isolation, characterization and mechanism of cytotoxic compounds from the *Boswellia dalzielii* stem bark.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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