

**Cytotoxic Activity of *Croton penduliflorus* (Euphorbiaceae) Seed Oil against Ehrlich Ascites Carcinoma Cells**Abiola S. Ojokuku<sup>1\*</sup>, Omolola S. Odesanmi<sup>2</sup>, Abiodun R. Lawal<sup>2</sup>, Abiola O. Magbagbeola<sup>2</sup>, Mehmet D. Ozaslan<sup>3</sup>, Didem I. Karagoz<sup>3</sup>, Halil I. Kilic<sup>3</sup>, Izzetin Guler<sup>3</sup><sup>1</sup>Department of Chemical Science, Yaba College of Technology, P.M.B. 2011 Yaba, Lagos, Nigeria.<sup>2</sup>Department of Biochemistry, College of Medicine, University of Lagos, P.M.B. 12003 Lagos, Nigeria.<sup>3</sup>Department of Biology, University of Gaziantep, 27310 Gaziantep, Turkey.

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## ABSTRACT

*Croton penduliflorus* (Euphorbiaceae) is a tropical medicinal plant ubiquitous to Nigeria and other West African countries. Its seeds are used in folklore medicine to treat uterine tumour and fibroid. This study was carried out to determine the cytotoxic effect of *Croton penduliflorus* seed oil (CPSO) on Ehrlich ascites carcinoma (EAC). The shelled, oven-dried, ground seeds of *Croton penduliflorus* was extracted with petroleum ether using Soxhlet apparatus to obtain the oil. Cytotoxicity of CPSO on EAC were carried out using trypan blue dye exclusion assay. A stock solution of 1000 µg/mL of CPSO in Dimethyl sulphoxide (DMSO) was prepared followed by serial dilutions of 100, 10 and 1 µg/mL from the stock. Aliquots of 200 µL of CPSO sample were placed in the wells and the volume were made up to 800 µL with DMSO. Aliquots of 100 µL of EAC with a concentration of 10<sup>6</sup> cells/mL was added to each well. Negative control having 800 µL DMSO in the wells followed by the addition of 100 µL of EAC with a concentration of 10<sup>6</sup> cells/mL were also prepared, while 5-Fluorouracil (5-FU) with concentrations of 1000, 100, 10 and 1 µg/mL was used as the positive control. The wells were incubated at 37°C for 24 h. Following the incubation, 100 µL of trypan blue was added to each well. The number of viable cells were counted in each well. CPSO has low cytotoxic effect on EAC with IC<sub>50</sub> of 561 µg/mL, while 5-FU has IC<sub>50</sub> of 2.88 µg/mL on EAC.

**Keywords:** *Croton penduliflorus*, Extract, Ehrlich Ascites Carcinoma, cytotoxicity.

## Introduction

Cancer is a broad term used to describe a series of malignant diseases that can affect different parts of the body. These diseases are usually caused by rapid and uncontrolled formation of abnormal cells which may lump together to form a growth or tumour or proliferate throughout the body imitating abnormal growth at other sites in the body. Cancer affects people of all ages, even fetuses and the risk for different forms increases with age.<sup>1-3</sup> Cancer is the second largest common diseases and a major global health burden. With changing life style and food habits coupled with exposure to various types of xenobiotics and radiations, also due to availability of curative treatments for many diseases. Cancer is surpassing other illnesses as a major cause of morbidity and mortality even in developing countries like Nigeria.<sup>4-6</sup> Cancer causes about 13% of all human deaths and according to the American Cancer Society around 7.6 million people die every year from cancer.<sup>7,8</sup>

Lots of cancer chemotherapeutics which are currently available affect the normal cells of patients very strongly as they have hepatotoxic, nephrotoxic, cardiotoxic, myelosuppressive and other side effects, and

have become serious medical problems.<sup>9</sup> This has stimulated the search for new medicaments from various sources, as a result there is a growing trend towards medicinal plants because of their perceived low toxicities and high medical effectiveness.<sup>10, 11</sup> Recently, eighty-eight medicinal plants with anti-tumour activity have been published by some scientists worldwide.<sup>12, 13</sup> A large number of chemotherapeutic agents have been developed in modern system of medicine from screening of medicinal plants in different part of the world.<sup>14, 15</sup> Ehrlich Ascites Carcinoma (EAC) has been reported to have a high transplantable capability, with no-regression, very rapid proliferation, shorter lifespan and 100% malignancy and does not have tumour-specific transplantation antigen (TSTA).<sup>16, 17</sup> In the past twenty years many researches have been carried out on this transplantable tumour. EAC is one of the commonest experimental tumour; it appeared first as a spontaneous breast cancer in female mouse and then Ehrlich. Apolant (1905) used it as an experimental tumour by transplanting tumour tissue subcutaneously from mouse to mouse.<sup>18, 19</sup> EAC has similarity with human tumours which are most sensitive to chemotherapy due to the fact that it is undifferentiated and it has rapid growth rate. Some researchers reported that some medicinal plants extracts were effective against EAC.<sup>20</sup> Chandra *et al*<sup>21</sup> reported IC<sub>50</sub> values of hexane, chloroform and dichloromethane extracts of *T. populnea* to be 38.94 µg/mL, 41.32 µg/mL, and 86.88 µg/mL against EAC cell line and hexane, chloroform and dichloromethane extracts of *H. schulli* to be 82.78 µg/mL, 83.84 µg/mL and 198.67 µg/mL against EAC. In their studies they concluded that hexane extracts of *T. populnea* and *H. schulli* were active against EAC.

Seed of *Croton penduliflorus* (Euphorbiaceae) commonly known as 'aworoso' in Yoruba land 'Ogwuaki' or 'Aki ozara' in Igbo land and 'koriba' in Hausa land. The plant grows from eastern Sierra Leone to Nigeria, Central African Republic to Gabon.<sup>22</sup> *Croton penduliflorus* seed constitute major components of herbal contraceptives,

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abortifacient and anti-fibroid concoctions used in the local treatment of uterine fibroids. *Croton penduliflorus* seed has also been used in folk medicine as a drastic purgative, emmenagogue, emetic, diaphoretic, rubefacient, antimicrobial, anti-venom, anti-scabies, anti-paralysis, anticancer remedies and as a stupefying bait in fishing.<sup>23, 24</sup> Acute toxicity studies on *Croton penduliflorus* seed oil showed that it is toxic to mice and rats.<sup>25</sup> The physicochemical properties, minerals and phytochemical constituents of *Croton penduliflorus* seed oil have been reported. The oil is acidic in nature, rich in linoleic acid (63.15%) and consist of other components like 1, 2 benzene dicarboxylic acid (5.2%), dibutyl phthalate (4.73%), cis-11-eicosenoic acid (3.55%) and gamma sitosterol (1.17%).<sup>26</sup> The effect of seed coat absence on the chemical composition of *Croton penduliflorus* seed oil has also been analyzed.<sup>27</sup> Despite its reported usage in folklore as anticancer, there is so far paucity of scientific information on its cytotoxicity. Therefore, this present research aims to determine the cytotoxic effect of *Croton penduliflorus* seed oil on Ehrlich Ascites Carcinoma (EAC) cell lines.

## Materials and Methods

### Chemicals

5-Fluorouracil (5-FU) was obtained from Kocak Farma (Turkey). Trypan Blue from Biotech PVT India, Dimethyl Sulphoxide (DMSO) from Sigma Aldrich. Other reagents used were of analytical grades.

### Plant material

*Croton penduliflorus* seeds were purchased from 'Alasalatu' herbal market, Mushin local government area of Lagos state, Nigeria. The sample was identified and authenticated by Professor Olowokudejo in the Department of Botany of the University of Lagos. A voucher specimen was deposited in the herbarium with reference number LUH 4531.

### Preparation of plant seed extract

*Croton penduliflorus* seeds were sorted cleaned and hard cover of the seeds coat removed. The seeds were oven dried at 45°C for three days and ground to powder with the Christy-Noris Laboratory Hammer Mill. The powdered seed sample was kept in an airtight container at room temperature (28°C) until extracted. Approximately 500 g of the powdered seeds was subjected to Soxhlet extraction with 2.5 L of Petroleum ether (40-60°C) into exhaustion. The liquid extracts were pooled, concentrated in a rotatory evaporator under reduced pressure and controlled temperature and oven dried at 40°C. The dried oil was stored in amber bottle and refrigerated until utilized.

### Cell lines

EAC cell lines were obtained from Molecular Biology laboratory, Department of Biology, Faculty of Arts and Science Gaziantep University, 27310 Gaziantep Turkey. They were maintained by weekly intraperitoneal inoculation of 10<sup>6</sup> cell/mouse.

### In-vitro Cytotoxicity of *Croton penduliflorus* seed oil

*In-vitro* cytotoxicity was carried out using Trypan blue dye exclusion method.<sup>28</sup> Petroleum ether extract of *Croton penduliflorus* seed was used for the preparation of the stock solution (1000 µg/mL) in DMSO. Serial dilutions (100, 10, and 1 µg/mL) were prepared with DMSO. Sample solutions (200 µL) were placed in wells and the volume in all the wells was made up to 800 µL with DMSO. 100 µL of EAC with a concentration of 10<sup>6</sup> cells/mL was added to the wells. A negative control having 800 µL DMSO in the wells followed by the addition of 100 µL of EAC with a concentration of 10<sup>6</sup> cells/mL was also prepared and a positive control of 800 µL of 5-Fluorouracil with concentrations of 1000, 100, 10 and 1 µg/mL were also prepared. The wells were incubated at 37°C for 24 h followed by the addition of 100 µL of Trypan blue (1%) to all the wells. The number of viable cells count was done in a Cedex cell counting machine (Roche, California). Results were expressed as percentage cell death.

$$\% \text{ dead cells} = \frac{\text{Number of dead cells}}{\text{Number of viable} + \text{number of dead cells}} \times 100$$

The IC<sub>50</sub> of the extract on EAC was calculated from the graph of percentage cell death of EAC and logarithm of concentrations of CPISO.

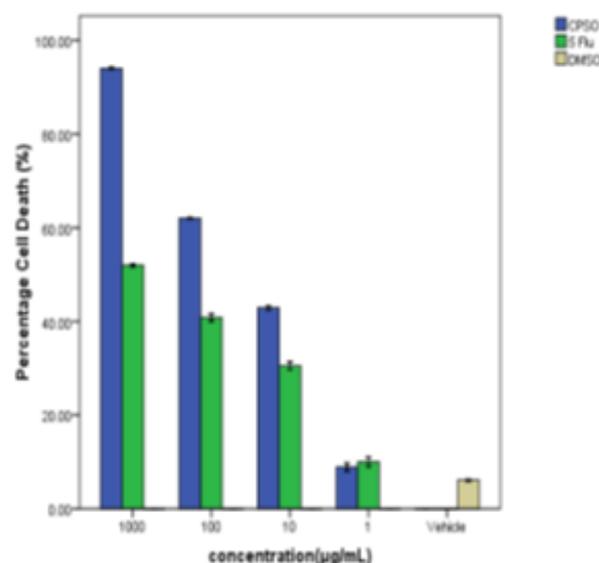
### Statistical analysis:

Tests were conducted in triplicate and statistical analysis was carried out using SPSS Version 18. Results were expressed as mean ± SEM. Graph pad Prism was used for the chart.

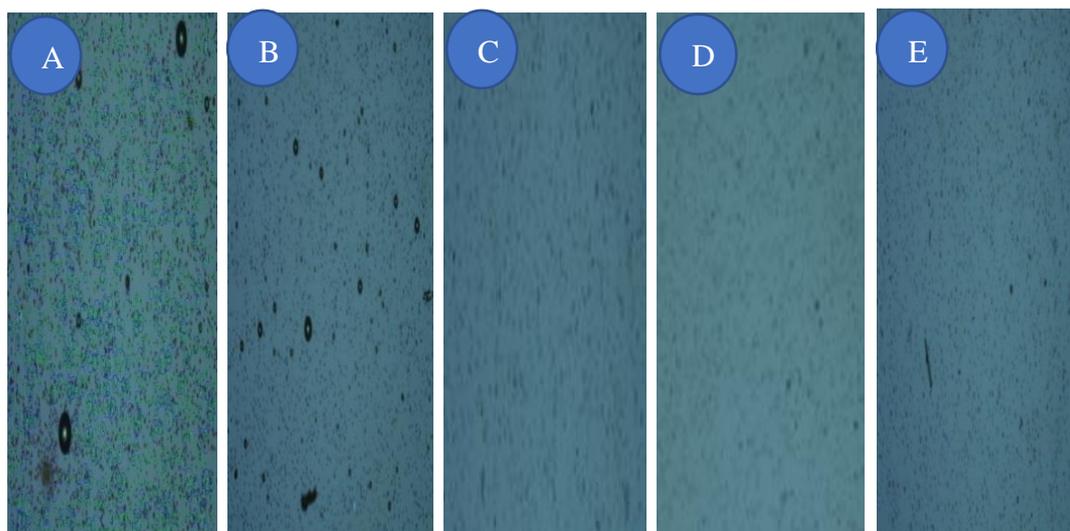
## Results and Discussion

Today scientists all over the world are working round the clock to combat cancer because treatment with orthodox drugs such as antimetabolites, antimitotic, antibiotic, topoisomerase inhibitors and others have severe side effects like leukemia, myelosuppression among others. Therefore, medicinal plants are useful economic resource and are potential source of new drugs for cancer.<sup>7, 28</sup>

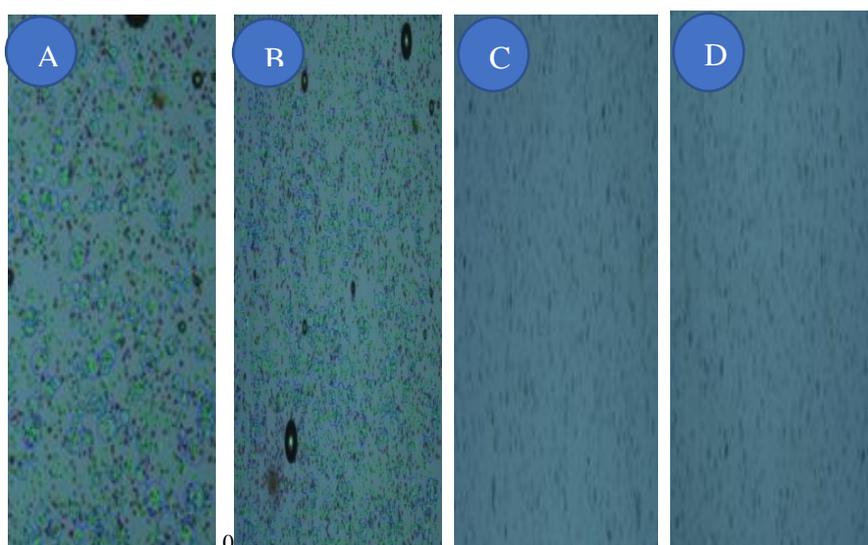
The plot of logarithm of concentrations of CPISO versus percentage cell death of EAC, gave IC<sub>50</sub> value of 561 µg/mL while 5-FU has IC<sub>50</sub> value of 2.88 µg/mL as shown in table 1. This indicates that CPISO has comparatively lower cytotoxic effect on EAC cell line than 5-fluorouracil. Morphological features of EAC stained with trypan blue dye after treatment with 1000 µg/mL of CPISO showed blue stains which indicate non-viable cells as shown in plate 1. Morphological features of EAC with 5-Fluorouracil showed non-viable cells compared to the control as shown in plate 2. Cytotoxicity or cell-based assays provide some basic information on plant extracts or compounds which affect cell viability.<sup>30, 31</sup> Most of the clinically used antitumour agents possess significant cytotoxic activity in cell culture and cytotoxic screening models which showcase germane preliminary data to select plant extracts or compounds with potential anti-cancer properties.<sup>21, 23</sup> *Croton penduliflorus* seeds are currently in use by traditional medicine practitioners to manage uterine fibroids in Nigeria. However, this study reveals that CPISO has low cytotoxicity towards Ehrlich Ascites Carcinoma cells with an IC<sub>50</sub> of 561 µg/mL while the positive control drug 5-Fluorouracil has a value of 2.88 µg/mL. This reference drug has been reported to increase significantly the life span of mice bearing EAC.<sup>34</sup> Crude extract is considered to have good cytotoxic activity when IC<sub>50</sub> is less than 20 µg/mL while pure compound with IC<sub>50</sub> less than 4 µg/mL is regarded as cytotoxic following 24-48 h incubation.<sup>35</sup> On the basis of this preliminary work, CPISO has low cytotoxic effects on EAC compared to other medicinal plant extracts whose IC<sub>50</sub> is less than 100 µg/mL.



**Figure 1:** *In-vitro* cytotoxic effects of *Croton penduliflorus* seed oil and 5-FU on Ehrlich ascites carcinoma after 24 hours of incubation.



**Plate 1:** Morphological features of EAC stained with trypan blue dye after 24 hours of incubation with (a) 1000 µg/mL of CPSO (b) 100 µg/mL of CPSO (c) 10 µg/mL CPSO (d) 1 µg/mL CPSO (e) DMSO (vehicle) Blue stained cells showed dead cells (non-viable cells) while unstained cells are viable.



**Plate 2:** Morphological features of EAC stained with trypan blue dye after 24 hours of incubation with (a) 1000 µg/mL 5-FU (b) 100 µg/mL 5-FU (c) 10 µg/mL 5-FU (d) 1 µg/mL 5-FU. Blue stained cells showed dead cells (non-viable) while unstained cells are viable.

### Conclusion

*Croton penduliflorus* seed oil has low cytotoxicity toward Ehrlich Ascites Carcinoma cells with  $IC_{50}$  of 561 µg/mL compared to 5-FU with  $IC_{50}$  of 2.88 µg/mL.

### Conflict of interest

The authors declare no conflict of interest.

### Author's 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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