



(RESEARCH ARTICLE)



CYTOTOXICITY OF AN ETHYL ACETATE EXTRACT OF *ANNICKIA POLYCARPA*

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ABSTRACT

Context: Medicinal plants represent constitute a promising avenue for the development of new therapies. They contain chemical compound capable of fighting cancer while inducing limited and tolerable side effects for patients. The aim of this study was to evaluate the cytotoxic potential of *Annickia polycarpa*, a plant used in traditional Ivorian medicine to treat solid tumors.

Methods: Cytotoxic activity of the extract was evaluated using MTS method on five human cancer cell lines. Doxorubicin was used as positive control.

Results: Among the tested cancer cells, HCT 116 were the most sensitive to the ethyl acetate extract of *A. polycarpa*, with an IC₅₀ of 1.61 ± 0.08 µg/mL.

Conclusion and outlook: Our results suggest that ethyl acetate extracts from *A. polycarpa* leaves represent an interesting candidate in the fight against colon cancer.

Keywords: *Annickia polycarpa*; Cytotoxic; Cancer Cell Lines; Traditional Ivorian Medicine

1 INTRODUCTION

In 2020, cancer was responsible for approximately 10 million deaths worldwide. It is the most widespread and lethal disease of our time [1] While surgery, chemotherapy, and radiotherapy remain the standard treatments, the latter two cause severe side effects for patients. To reduce this toxicity, developing new approaches is essential. Phytotherapy represents a promising alternative, as plant-derived compounds offer great potential for creating better-tolerated cancer therapies.

Among many others, we can cite a study conducted by [2] in which it was found that the plants *Artemisia absinthium*, *Hyoscyamus niger* and *Plantago major* exhibit toxicity against lung, breast and prostate cancers.

In Côte d'Ivoire, during an ethnopharmacological survey, several plants were cited as treatments against cancer. Among them is *Annickia polycarpa*, a plant belonging to the Annonaceae family. The main objective of the present work was to evaluate the cytotoxic potential of ethyl acetate extracts of *A. polycarpa* leaves on five human cancer cell lines.

2 MATERIALS AND METHODS

2.1 Cell lines and culture conditions

All cell lines utilized in this study were of human origin and obtained from American Type Culture Collection (ATCC) (Manassas, Virginia). The culture media, fetal calf serum, trypsin and antibiotics were purchased from Sigma-Aldrich (St Louis, Missouri). HCT 116 (colorectal carcinoma, ATCC-CRL-3488), MDA-MB-231 (breast adenocarcinoma, ATCC HTB-26MET that does not express estrogen or progesterone receptors), MCF-7 (breast adenocarcinoma, ATCC HTB-22 that expresses estrogen receptor), PC3 (prostate carcinoma, ATCC CRL-1435) are adherent epithelial cells. THP-1 (monocytic leukaemia ATCC TIB-202) are derived from acute monocytic leukemia and are maintained in suspension. Culture medium RPMI 1640 medium (Sigma-Aldrich R2405-500mL) was utilized for HCT 116, MDA-MB-231 and THP-1; DMEM/F12 medium (PAN Biotech P04-41550-500mL) was employed for PC3; and DMEM-high glucose medium (Sigma-Aldrich D0819-500mL) was used for MCF7. All basal media were supplemented with 10 % (V/V) fetal bovine serum and 1 % penicillin-streptomycin (P/S: 10 U/0.1 mg). The cells were incubated at 37 °C in a humidified atmosphere with 5 % CO₂.

2.2 Plant material

Leaves of *A. polycarpa* were collected in Daloa, Côte d'Ivoire, during February 2023. The botanical identification was carried out at the National Floristic Center of Abidjan. The herbarium number assigned to the plant is UCJ001189. Following the harvesting process, the leaves were dried in a shade environment at room temperature. The material was then pulverized and meticulously preserved.

2.3 Preparation of extracts

The plant powder was subjected to extraction. Twenty grams of powdered leaves were macerated in 250 mL of ethyl acetate for a period of 24 hours. The resulting extract was filtered on Whatman paper, and the solvent was removed using a rotary evaporator until a dry extract was obtained. The crude extract was stored at -20°C for future use. The percentage yield of extraction was calculated using the following formula [3]:

$$R = \frac{mf}{mi} \times 100$$

R: yield (%)

mf: final mass of extract (gram)

mi : initial mass of extract (gram)

2.4 Evaluation of the cytotoxicity by MTS

This method entails the evaluation of mitochondrial activity and by extension cell viability following treatment with a product using the CellTiter 96® AQueous One Solution Reagent kit (Promega, Madison, Wisconsin) in accordance with the manufacturer's instructions. 20,000 to 50,000 cells according to the cell line were seeded per well in 96-well plates and incubated at 37°C with 5 % humidified CO₂ for 24 hours.

10 mg of the extract was dissolved in a mixture of dimethyl sulfoxide and distilled water (1:1) to obtain a stock solution with a concentration of 10 mg/mL. The extracts were then diluted in a complete culture medium. The cells were treated with a concentration range from 200 µg/mL to 0.001 µg/mL. The ranges of dimethyl sulfoxide were prepared in the same serial dilution as for the extracts but from the DMSO/H₂O solution.

Doxorubicin, a reference anticancer agent, was used at 1 µM as a positive toxicity control. Following treatment, the cells were observed under a microscope and then returned to the incubator for 24 hours before the MTS test. Then, after removal of the cell supernatant, 100 µL of a MTS/PBS mixture (1:9) was added to the wells, including into empty wells (i.e. without cells = the blank). After approximately one hour (until the staining was sufficiently intense), the absorbance of the plates was measured at 490 nm using a spectrophotometer. The absorbance intensity is proportional to the number of live cells. The results were expressed as percentage cell viability using the following formula [4]

$$\% \text{ Viab} = \frac{A_{\text{sample}} - A_{\text{white}}}{A_{\text{witness}} - A_{\text{white}}} \times 100$$

% Viab: Percentage of cell viability

A_{Sample}: Absorbance of the treated cells tested (extracts)

A_{Witness}: Absorbance of the untreated cells

A_{White}: Absorbance of blank (MTS without cells).

All experiments were conducted in technical triplicates (three wells/concentration, intra-experiment reproducibility) and then in biological triplicates (n=3, inter-experiment reproducibility). The results are expressed as the mean ± standard error of the mean. The sigmoid dose-response curve was obtained from non-linear regression analyses (logarithm of inhibitor versus normalized response) using GraphPad Prism software (version 8) and used to determine the inhibitory concentration at 50 % (IC₅₀).

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3 RESULTS

The extraction carried out from 20 g of powder using ethyl acetate allowed us to obtain a yield of 5.24 %. The extract was then tested on five Human cancer cell lines. Its cytotoxicity was assessed by the percentage of live cells in the presence of a well-defined range of extract concentrations from 200 µg/mL to 0.001 µg/mL. The MTS test was performed one day after addition of the extracts. The results showed that the extract was toxic to all cell lines with IC₅₀ ranging from 1.61 to 169 µg/mL (Table 1). The doxorubicin used at 1 µM resulted in an IC₅₀ of 0.5 µg/mL.

Table 1: Inhibitory concentration values IC₅₀ of the *E. polycarpa* (in µg/mL)

Extract	HCT 116	MCF-7	MDA-MB-231	THP-1	PC3
<i>E. polycarpa</i>	1.61 ± 0.08	85.34 ± 0.04	71.84 ± 0.6	169.72 ± 0.51	65.07 ± 0.06

The lowest IC₅₀ has been obtained on HCT 116 cells (IC₅₀ = 1.61 ± 0.08 µg/mL). On these latter cells the cytotoxic effects started already at 0.01 µg/mL and viability decreased to > 90 % at 200 µg/mL (Figure 1).

In MDA-MB-231, MCF-7, two breast cancer cell lines and THP-1, a monocytic cell line, toxicity started above 20 µg/mL (70% viability) (Figures 2, 3 and 4). However, it should be noted that the toxicity of the DMSO/H₂O control was also significant at the 200 µg/mL point, especially in THP-1 and MDA-MB-231, indicating that the DMSO concentration was already too high for the cells. Finally, on PC3, a prostate cancer line, the toxicity was seen at 70 µg/mL and increased up to 200 µg/mL (10 % viability) (Figure 5).

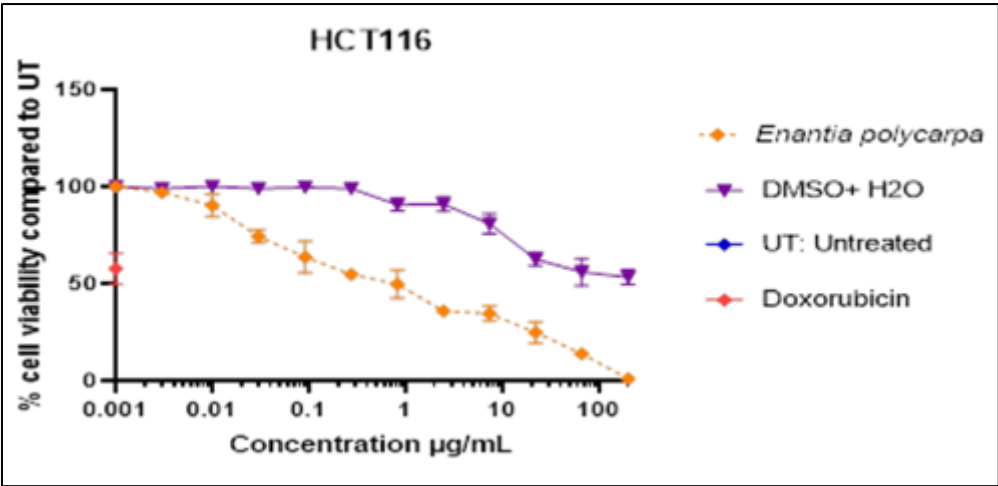


Figure 1: Dose-response activity of *A. polycarpa* leaves extracts on HCT 116 cells.

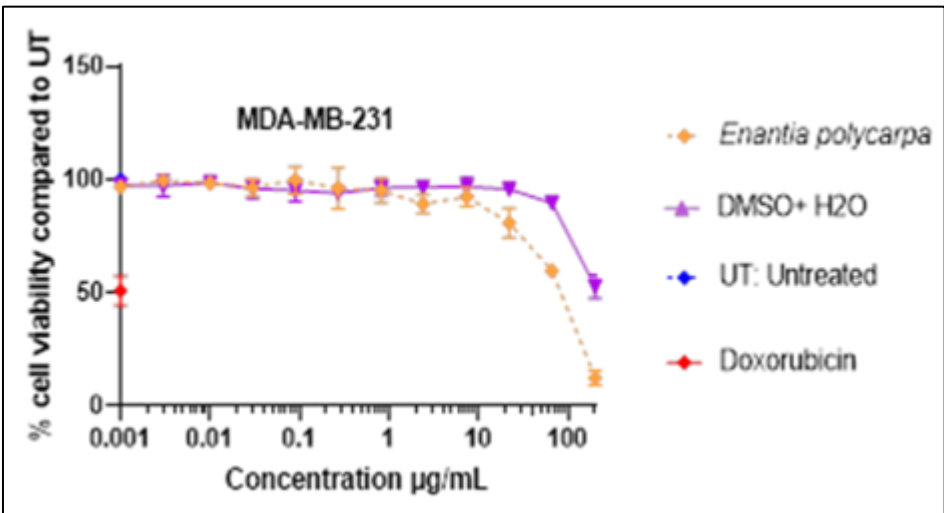


Figure 2: Dose-response activity of *A. polycarpa* leaves extracts on MDA-MB-231 cells.

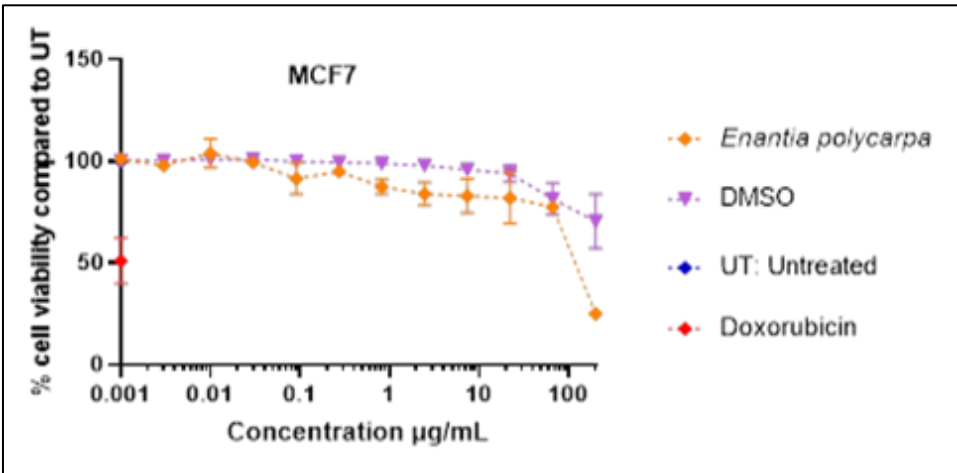


Figure 3: Dose-response activity of *A. polycarpa* leaves extracts on MCF-7 cells.

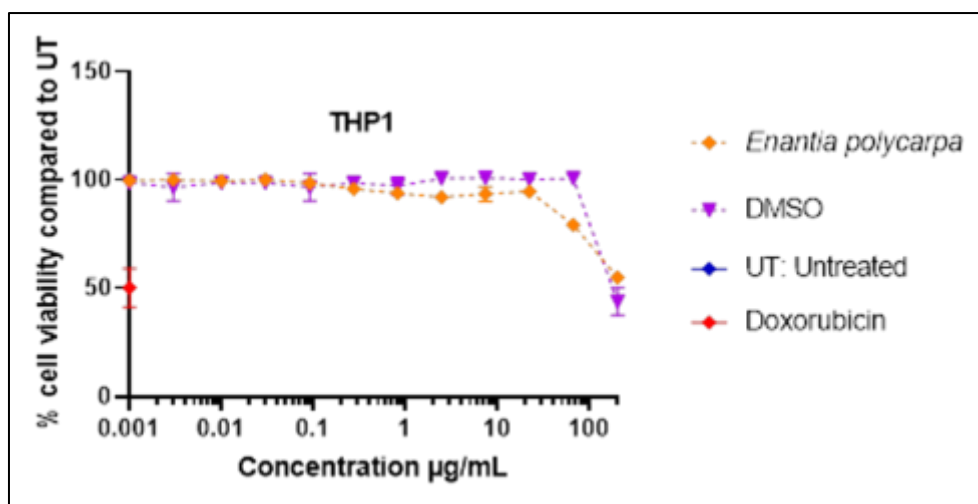


Figure 4: Dose-response activity of *A. polycarpa* leaves extracts on THP-1 cells.

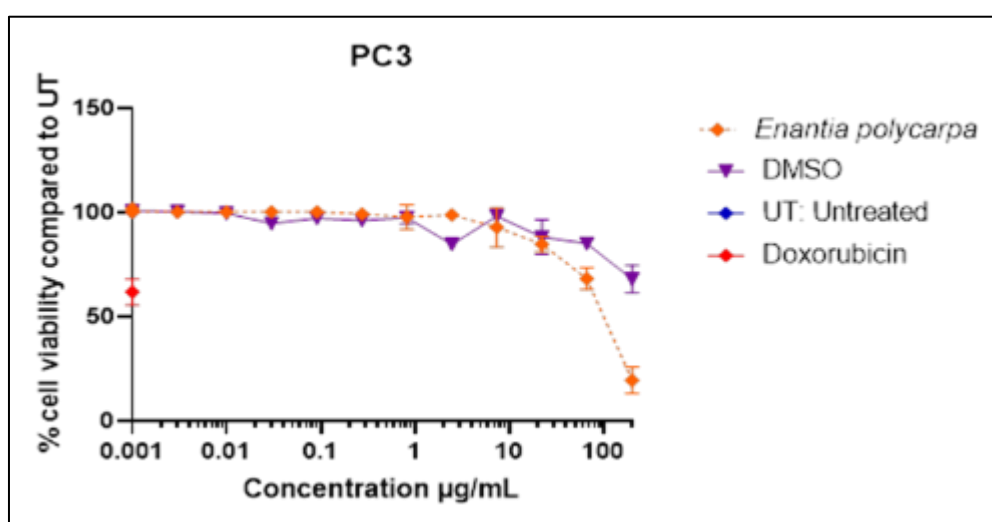


Figure 5: Dose-response activity of *A. polycarpa* leaves extracts on PC3 cells

4 DISCUSSION

The studied plant has been frequently cited in various ethnobotanical studies due to its traditional use in the treatment of tumors [5]. It also offers the advantage of being widely available and easily accessible. Nevertheless, despite its widespread empirical use, its pharmacological properties remain largely unexplored and poorly documented, which fully justifies the interest of this study.

Ethyl acetate was selected as the extraction solvent because of its low toxicity and its ability to effectively isolate a broad spectrum of secondary metabolites.

Our results indicated that the leaf extract exhibited cytotoxicity against a variety of Human tumor cells, including HCT 116 cells, MDA-MB-231, MCF-7, THP-1, and PC3 cell lines - its effects on THP-1 cells was however minimal.

The analysis of IC_{50} values from ethyl acetate extract of the studied plants reveals a variability in toxicity depending on the targeted cell line. According to the criteria established by the United States National Cancer Institute (NCI), the cytotoxicity of crude extracts is interpreted using a rigorous scale: an IC_{50} value below 20 $\mu\text{g/mL}$ is considered highly toxic, between 20 and 100 $\mu\text{g/mL}$ indicates moderate toxicity, a range from 100 to 500 $\mu\text{g/mL}$ corresponds to low toxicity, and a value above 500 $\mu\text{g/mL}$ is regarded as indicating no significant toxicity [6] [7] [8]). This classification thus allows for an assessment of the diversity of cytotoxic effects observed across different extracts and cell types.

Thus, on the HCT 116 cell line, the ethyl acetate extract of *Annickia polycarpa* ($IC_{50} = 1.61 \pm 0.08 \mu\text{g/mL}$) exhibits high cytotoxicity. In contrast, on the THP-1 cell line, the extract shows low toxicity. For the MDA-MB-231, MCF-7, and PC3 cell lines, the extract displays moderate toxicity. In comparison, the study by [9] reports that the ethyl acetate extract of *Selaginella doederleinii* presents an IC_{50} of $20.13 \pm 3.11 \mu\text{g/mL}$ on HCT 116 cells, which corresponds to moderate toxicity. These findings suggest that *E. polycarpa* has stronger cytotoxic activity than *S. doederleinii*.

The moderate effects observed with *Annickia polycarpa* extracts on the MDA-MB-231 (hormone-independent breast cancer), MCF-7 (hormone-dependent breast cancer), and PC3 cell lines suggest that this plant may act on both hormonal and non-hormonal tumor mechanisms. It may be capable of inhibiting or modulating the activity of hormones involved in the proliferation of cancer cells, as demonstrated with the MCF-7 and PC3 lines [10]. The results obtained in this study appear more promising than those reported by [11] who found low toxicity ($IC_{50} = 467 \mu\text{g/mL}$) of the ethyl acetate extract from *Cakile arabica* leaves on the MDA-MB-231 cell line.

The efficiency of our extract is rather interesting as compared to other extracts: indeed, on MCF-7 cells, our extract demonstrates greater efficacy than the ethyl acetate extract of *Krameria lappacea* studied by [12] who reported an IC_{50} of $706 \mu\text{g/mL}$.

5 CONCLUSION

This study, which focused on the anti-cancer properties of *A. polycarpa* against colon, breast, blood, and prostate cancer cells, is part of a broader research program aimed at discovering new anti-cancer molecules of plant origin. The results show that *A. polycarpa* has significant cytotoxic activity, especially against colon cancer cells ($IC_{50} = 1.61 \pm 0.08 \mu\text{g/mL}$).

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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