Original Research Article

Cytotoxicity activity and Phytochemical Screening of *Anthocleista djalonensis* Root extracts against Cancer.

ABSTRACT

Aim:~~Every part of~~ *Anthocleista djalonensis* ~~-~~various ~~has been reported for~~ medicinal uses have been reported for the whole plant and ~~as they all have various therapeutic values with~~ many ~~kinds~~ types of pure compounds have been isolated. However, the anti-cancer ~~in~~ of this plant has not been proven. The aim of this study ~~is~~ was to screen for the phytochemicals present in the root *n*-h~~H~~exane, ethyl acetate, and acetone extracts ~~of root~~ of *Anthocleista djalonensis*, and to evaluate its anticancer potential against human cervix adenocarcinoma cells (HeLa cells) *in vitro*.

Place and duration of study: The study was carried in department of Organic Chemistry, Rhodes University, Grahamstown, South Africa. The duration period was between March – July, 2016.

Methodoogy: Extracts were prepared by allowing the root powder to ~~react~~ soak in the ~~with~~ respective solvents with continuous agitation; it was then filtered and condensed. The extracts were then screened for ~~its~~ phytocompounds by preliminary screening methods. Anti-cancer potential was detected by resazurin assay using 7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide (resazurin) reagent and CC_{50} values were claculated.

Results: The extracts revealed the presence of Carbohydrates, Glucoside, Alkaloids, Flavonoids, Terpenoids, Tannins, Sapoonin, Sterols. All extracts demonstrated moderate cytotoxicity against HeLa cells.

Conclusion: Hexane, Ethyl acetate and acetone extracts showed anticancer property. The

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roots extracts of *Anthocleista djalonesis* was thus found to possess anticancer potential.

Keywords: ***Anthocleista djalonensis*, Anti-cancer, Cytotoxicity, HeLa cells, Phytochemicals, Resazurin assay**

1. INTRODUCTION

Cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015 [1] Globally, nearly 1 in 6 deaths is due to cancer [2].There has been an intense search on various biological sources to develop a novel anti-cancer drugs to combat this disease. Plants have proved to be an important natural source of therapeutic agents. Medicinal plants contain chemical substance or constituents that have pharmacological activities [3].These activities include anti-cancer, anti-tumor, anti-oxidant and anti-microbial activities [4, 5, 6]. In view of the reported adverse effects of orthodox anticancer drugs [7,8,9], and the confirmed efficacy of medicinal plants [10.11,12,13,14], there is need to continuously search for plant-derived anticancer agents. *Anthocleista djalonensis* is one of those plants that are used traditionally for the treatment of several diseases like cough, tuberculosis, jaundice, etc. Recently, Ethnobotanical investigation revealed the use of *Anthocleista djalonensis* for the treatment of cancer [15]. However, the anti-cancer in this plant has not been proven [...list the many types of pure compounds that have been isolated with their citations]..... This study is was carried out in orderas an attempt to scientifically validate the cytotoxic effect of *A. djalonensis* root hexane, ethyl acetate and acetone extracts against Cancer cells human cervix adernocarcinoma (HeLa) cells. This will be of tremendous assistance in assessing the safety of the medicinal plants and also give direction for future anticancer drug development.Rephrase this sentence

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2. MATERIAL AND METHODS

2.1. Collection of plant material

The root of *Anthocleista djalonensis* was obtained from Zakibiam in Benue State. The plant taxanomic identification was established by Mr Ibe Ndukwe of the Forestry department, Michael Okpara University of Agriculture Umudike. Voucher samples of the plants are deposited in the Herbarium of Michael Okpara University of Agriculture Umudike, Nigeria. The roots were dried under a shade for three weeks and were milled at the Chemistry Department, University of Agriculture Makurdi using Thomas model 4 Willey Mill.

2.2 Extraction of plant material

The ~~puverised~~pulverized plant materials (1200 g for *Anthocleista djalonensis*) ~~was~~ were macerated in methanol for one week and concentrated on a rotary evaporator at 35 ^{0}C separately. TLC was done on the concentrates obtained to give a combined thick residue of 93.61 g for *A. djalonensis* (light brown colour).

2.2.1. Maceration of crude extract

The 93 g of crude extract was extracted successively with ~~-~~hexane (4 x 100 mL), ethyl acetate (4 x 100 mL) and acetone (4 x 100 mL) by maceration. The extracts were concentrated individually ~~with~~ using rota vapor.

2.3. Phytochemical Screening

Phytochemical screening of the crude extract was carried out employing standard procedures [16].

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2.4. HeLa Cell culture and treatment [17]

Human cervix adenocarcinoma cells (HeLa) ~~obtained~~ (~~from~~ ATCC CCL-2 LGC standard Wesel, Germany) were cultured in a 5% CO_2 incubator at 37^{o}C in DMEM medium supplemented with 10% fetal bovine serum and antibiotics

(penicillin/streptomycin/fungizone). The cells were split every 3-5 days (when the cells ~~have~~ had reached close to full confluency): the cells were detached from the culture flask surface using trypsin/EDTA, and the majority aspirated off. Medium was added to the flask and the remainder of the cells, and the flask returned to incubation. The confluency and state of the cells ~~was~~ were regularly assessed using an inverted light microscope. Cells ~~was~~ were cryopreserved by detaching the cells from the culture flask in trypsin/EDTA, pelleting the cells, transferring them to cryotubes in 10% DMSO in fetal bovine serum, and placing the tubes in a -80 freezer. For the cytotoxicity assay a range of concentrations of extract (1-250 µg mL^{-1}) ~~was~~ were used for 24 h treatment for the determination of CC_{50}.

2.5. *In vitro* Cytotoxicity Assay

In vitro ~~Cytotoxic~~ cytotoxic activity was determined by resazurin reduction based assay [18] HeLa cells were used for the determination of the CC_{50} value of the cytotoxicity of the ~~*Pycnanthus angolensis* stem bark extracts~~ Check?. To assess the cytotoxicity of the compounds, extracts were incubated at various concentration ~~of~~ in 96-well plates containing HeLa ~~(human cervix adenocarcinoma)~~ cells for 24 hours. The number~~s~~ of cells surviving the drug exposure were also determined by using the resazurin based reagent and reading resorufin fluorescence ~~in~~ using a multiwell plate reader. Reagents ~~was~~ were prepared by dissolving high purity resazurin in DPBS (pH 7.4) to 0.15 mg/mL. The resazurin solution was filtered and sterilized through a 0.2 µm filter into a sterile, light protected container. The resazurin solution was stored and protected from light at 4 °C for frequent use or at -20 °C for long term storage. Cells and test compounds were prepared in opaque-walled 96-well plates containing a final volume of 100 µL/well. An optional set of wells were prepared with

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medium only for background subtraction and instrument gain adjustment. This was incubated for the desired period of exposure. Twenty µl resazurin solution was added to each well. This was incubated for 1 to 4 hours at 37 °C. The fluorescence was recorded using a 560 nm excitation / 590 nm emission filter set.

2.6. Analysis of data

Quantitative values obtained ~~per treatment~~ were converted to percentage cell viability. Regression analysis was used to compute the percentage cell viability concentration required to produce a 50% reduction in cell viability (CC_{50}). Results were expressed as the mean ± SD of values obtained in triplicate ~~from~~ for three independent experiments. Statistical differences between correlated samples were evaluated using Student's *t*-test and noted to be significantly different where $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening of *A.djalonensis* root extract

The phytochemical screening of hexane, ethyl acetate and acetone extracts showed the presence of –Carbohydrates, Glycosides, Alkaloids, Flavonoids, Terpenenoids, Tannins, Saponins and Sterols. The results and observations are summarized in Table 1.

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Table 1: Phytochemical screening of extracts

Fraction	Carbo-hydrates	Gly-cosides	Alkaloids	Flavonoids	Terpenoids	Tannins	Saponins	Sterols
Hexane	+	+	+	+	+	-	-	+
Ethyl acetate	+	+	+	+	+	+	-	+
Acetone	+	+	+	+	+	+	+	+

+ =Presence, - = Absence

3.2. Cytotoxicity assay

The cancer cell viability of hexane, ethyl acetate and acetone extracts are presented in Fig 1-3. The percentage cell viability ~~increased~~ with respect to the concentration. The CC_{50} values for hexane, ethyl acetate and acetone were 241 ug/mL, 170ug/mL and 97 ug/mL respectively. The acetone extract demonstrated the highest activity while hexane and ethyl acetate extracts showed low activity against HeLa cells. Their ~~potent~~ cytotoxic effect may be considered for further evaluation using other cell types, especially the acetone extract which was capable of inducing cytotoxicity down to CC_{50} < 100 ug/mL.

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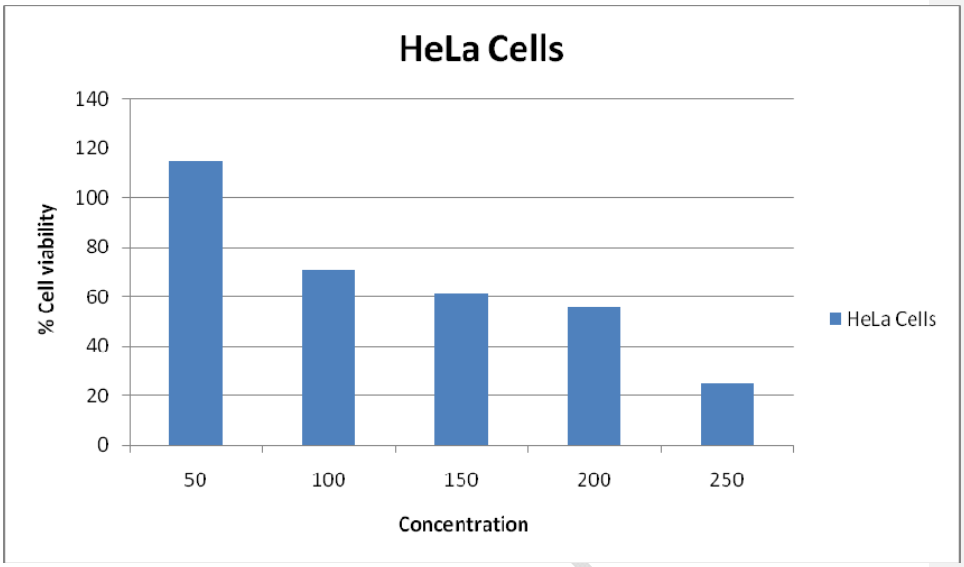

Fig. :1 Cytotoxicity effect of hexane extract against HeLa cells at different concentration (ug/mL)

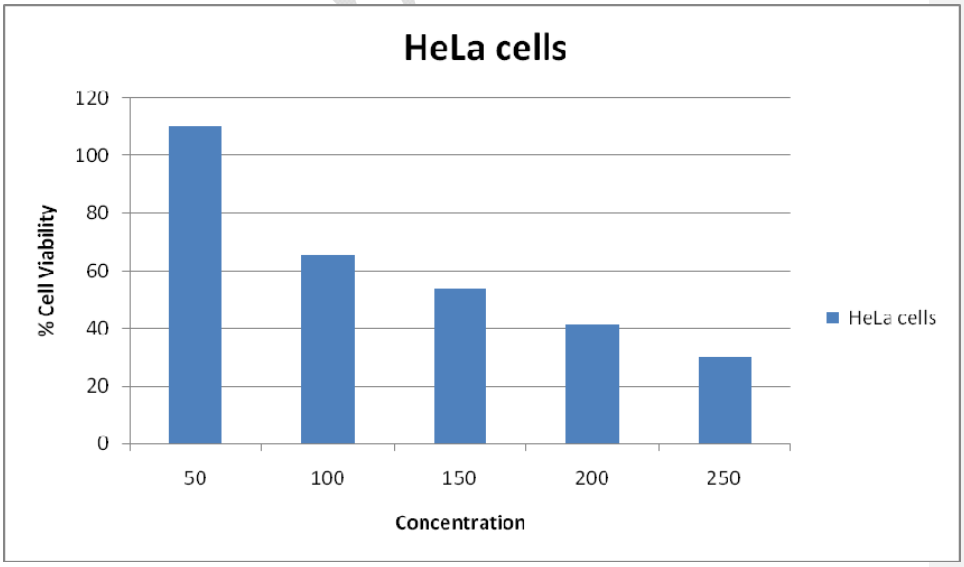

Fig. 2: Cytotoxicity effect of ethyl acetate extract against HeLa cells at different concentration (ug/mL)

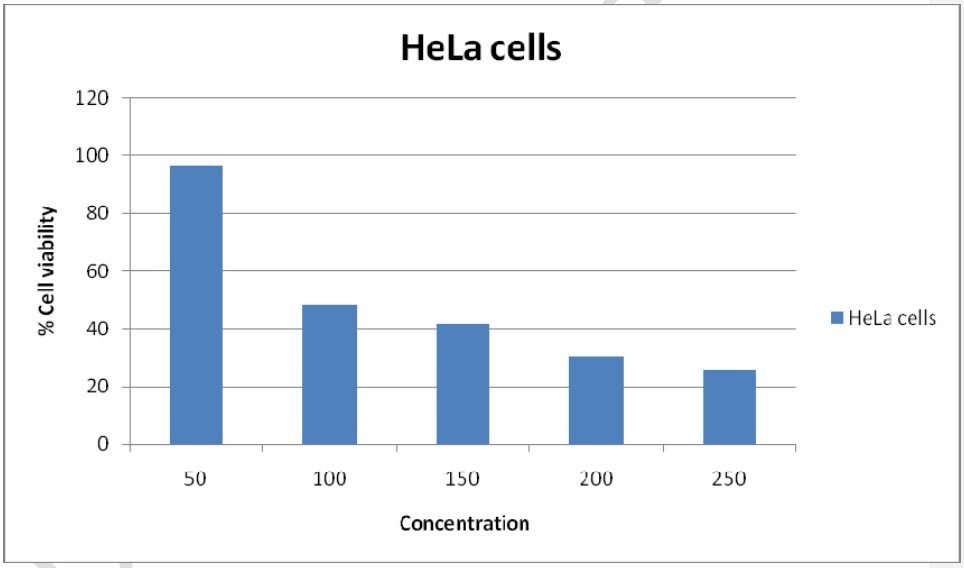

Fig 3: Cytotoxicity effect of acetone extract against HeLa cells at different concentration(ug/mL

Table 2: The CC_{50} of Extracts against Hela Cells

Fraction	Cytotoxicity(CC_{50})
Hexane	**241**
Ethyl acetate	**170**
Acetone	**97**
Emetine	**0.01049**

3.3. Discussion

Plants and plant derived products have proved effective and safe in the treatment and management of cancers [19]. Phenols and flavonoids are phytochemicals found in plants that have good anticancer potentials with considerable effect on human nutrition and health (20, 21, 22, 23). The identification of anticancer agents from plants is a consistent and continuous process. The present study was carried out in order to screen *in vitro* cytotoxic activities of *Anthocleista djalonensis* root extract ~~on~~ against HeLa cells. The extracts exhibited moderate cytotoxicity (32 to 499) in accordance to classification by Abdul et al., [24]. Acetone root extract demonstrating the highest cytotoxicity with ethyl acetate root extract being the lowest. The activities ~~demonstrated~~ varied according to ~~by~~ the different polarity of extracts at different concentration may be attributed to the uneven distribution of phytochemicals within these extracts. The activity of these extracts against HeLa cells ~~is in confirmation~~supported ~~of~~ the ethnobotanical use of ~~the~~ *Anthocleista djalonensis* in cancer treatment as reported above [ref]. The acetone extract ~~with~~ exhibited the highest cytotoxicity (CC_{50} < 100 units) contains the maximum number of bioactive chemicals which could be responsible for its cytotoxic effect. Chemical constituents reported in this study from the extracts were ~~Carbohydrates~~carbohydrates, ~~Glycosides~~glycosides, ~~Alkaloids~~alkaloids, ~~Flavonoids~~flavonoids, ~~Terpenoids~~terpenoids, ~~Tannins~~tannins, ~~Saponins~~saponins, ~~Sterols~~sterols. Awah et al., [25] reported phenolic compounds and ~~Flavonoids~~ flavonoids as being a major class of bioactive components in *Anthocleista djalonensis* plant. These biologically active compounds may be responsible for the *in-vitro* cytotoxic activity of root

extract against the HeLa cell lines. The ~~determination of~~extract cytotxicity was carried out in comparison ~~with~~ to the Emetine (positive control). Emetine demonstrated a higher activity with CC_{50} value of 0.01049 µg/ml. ~~The root extracts in their crude form may not have shown a very high activity possibly because of lack of inducer of the inhibitor. Thus, the actual activity of an active principle of extract can only be highlited by purification of the crude.~~

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4. CONCLUSION

This present study reveals the extracts of *A. djalonensis* as a ~~good~~ potential source of natural anticancer agents. The result showed potent cytotoxic activity against HeLa cell line for all extracts. Further *in vitro* and *in vivo* with different human cell lines study is required to demonstrate the anticancer and antitumor activity of this plant. Further isolation and identification of the active compounds as lead in the extracts is recommended for the drug development.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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