European Journal of Medicinal Plants

27(2): 1-7, 2019; Article no.EJMP.36932
ISSN: 2231-0894, NLM ID: 101583475

Cytotoxicity Activity and Phytochemical Screening of *Anthocleista djalonensis* Root Extracts against Cancer Cells

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Authors' contributions

This work was carried out in collaboration among all authors. Author ISO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JOI and MEK managed the analyses while author TATA managed the literature search. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2019/v27i230108
Editor(s):
(1) Dr. Shanfa Lu, Professor, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, China.
(2) Dr. Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) O. Mazimba, Chemical and Forensic Sciences, BIUST, Botswana.
(2) Wagner Loyola, Brazilian Agricultural Research, Brazil.
Complete Peer review History: http://www.sdiarticle3.com/review-history/36932

Original Research Article

Received 22 July 2017
Accepted 19 October 2017
Published 30 March 2019

ABSTRACT

Aim: Several medicinal uses have been reported for *Anthocleista djalonensis* and many types of pure compounds have been isolated. However, the anti-cancer activity of this plant has not been proven. The aim of this study was to screen for the phytochemicals present in the root*n*-hexane, ethyl acetate, and acetone extracts 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 out in Department of Organic Chemistry, Rhodes University, Grahamstown, South Africa. The duration period was between March and July, 2016.
Methodology: Extracts were prepared by soaking the root powder in the respective solvents with continuous stirring; The extracts were filtered and evaporated to remove the solvents. The extracts were then screened for phytocompounds by preliminary screening methods. Anti-cancer potential was carried out by a Resazurin assay and CC_{50} values were determined.

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Results: The extracts showed the presence of carbohydrates, glucoside, alkaloids, flavonoids, terpenoids, tannins, saponins, sterols. All extracts demonstrated moderate cytotoxicity against HeLa cells.
Conclusion: The hexane, ethyl acetate and acetone extracts showed anticancer property. The roots extracts of *Anthocleista djalonesis* were thus found to possess potential anticancer activities.

Keywords: Anthocleista djalonensis; anticancer; cytotoxicity; HeLa cells; phytochemicals; Resazurin assay.

1. INTRODUCTION

Cancer is the second leading cause of death globally after Ischaemic heart disease 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 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 *Anthocleistadjalonensis* for the treatment of cancer [15]. However, the anti-cancer in this plant has not been proven. Iribacholine [16], monoterpene-dioldjalonenol,dibenzo-pyronedjalonensone, djalonenoside [17], amplexine and axanthone lichexanthone [18] are compounds isolated from the roots of *Anthocleistadjalonensis*. Phthalide djalonensin has been reported to contain in the stem bark [18]. This study was carried out as an attempt to scientifically validate the cytotoxic effect of *A. djalonensis* root hexane, ethyl acetate and acetone extracts against human cervix adernocarcinoma (HeLa) cells. Whereby the safety of the plant is guaranteed and the direction for future anticancer drug development is ascertained.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The roots of *Anthocleista djalonensis* were obtained from ZakiBiam in Benue State. The plant was identified by Mr Ibe Ndukwe of the Forestry Department, Michael Okpara University of Agriculture Umudike. A voucher specimen Specimen No: AD/124 of the plant was deposited in their Herbarium. The roots were dried under a shade for three weeks and were powdered using a Thomas model 4 Willey Mill at the Chemistry Department, University of Agriculture Makurdi.

2.2 Extraction of Plant Material

The extraction was carried out as describe by Okoro et al. [19]. A light brown colour residue of 93.61 g for *A. djalonensis* was obtained.

2.2.1 Maceration of crude extract

The protocol for maceration as described by Okoro et al. [19] was adopted. Rota vapor was used to remove the solvents to obtain hexane, ethyl acetate and acetone extracts.

2.3 Phytochemical Screening

Phytochemical screening of the crude extract was carried out employing standard procedures as adopted [20].

2.4 HeLa Cell Culture and Treatment [21]

A 5% CO_2 incubator at 37°C in DMEM medium supplemented with 10% fetal bovine serum and antibiotics (penicillin/streptomycin/fungizone). Was suitable for culturing the human cervix adenocarcinoma cells (HeLa) obtained from ATCC CCL-2 LGC standard Wesel, Germany, When the cells had reached close to full confluency (every 3-5 days), the cell will split. This was done by using trpsin/EDTA to detach cells from the flask allowing the majority to aspirat off. Medium was added to the culture flask and the remainder of the cells, and the flask returned to incubation.The assessment of the confluency and state of the cells was performed regularly usingan inverted light microscope. Cells 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 determination of CC_{50}, a range of concentrations of extract (1-250 µg mL^{-1}) were used for 24 h treatment.

2.5 *In vitro* Cytotoxicity Assay

In vitro cytotoxic activity was determined by a resazurin reduction based assay as described by Okoro et al. [19]. HeLa cells were used for the determination of the CC_{50} value of the cytotoxicity of *Anthocleistadjalonensis*. To assess the cytotoxicity of the compounds, extracts were incubated at various concentration in 96-well plates containing HeLa cells for 24 hours. The number of cells surviving the drug exposure was also determined by using the resazurin based reagent and reading resorufin fluorescence using a multiwell plate reader. Reagents 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 medium only for background subtraction and instrument gain adjustment. This was incubated for the desired period of exposure. 20 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 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 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.

3.2 Cytotoxicity Assay

The cancer cell viability of hexane, ethyl acetate and acetone extracts are presented in Figs. 1-3. The percentage cell viability decreased with respect to the increase in concentration. The CC_{50} values for hexane, ethyl acetate and acetone were 241.01± 3.97 µg/mL, 170.02 ± 1.93 µg/mL and 97.00 ± 1.26 µg/mL respectively. The acetone extract demonstrated the highest activity while hexane and ethyl acetate extracts showed low activity against HeLa cells. The significant ($P<0.05$) cytotoxicity 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.

3.3 Discussion

Plants and plant derived products have proved effective and safe in the treatment and management of cancers [22]. Phenols and flavonoids are phytochemicals found in plants that have good anticancer potentials with considerable effect on human nutrition and health [23-26]. The identification of anticanceragents from plants is a consistent and continuous process. The present study was carried out in order to screen *in vitro* cytotoxic activities of *Anthocleistadjalonensis*root extract against HeLa cells.The extracts exhibited moderate cytotoxicity (32 to 499) in accordance to classification by Abdul et al. [27]. Acetone root extract demonstrating the highest cytotoxicity with ethyl acetate root extract being the lowest. The activities varied according to 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 supported by the ethnobotanical use of *Anthocleista djalonensis* in cancer treatment as reported above [15]. The acetone extract exhibited the highest cytotoxicity ($CC_{50}<$ 100 units). Thus 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, glycosides, alkaloids, flavonoids, terpenoids, tannins, saponins, sterols. Awah et al. [28] reported phenolic compounds and

Table 1. Phytochemical screening of extracts

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

+ =Presence, - = Absence

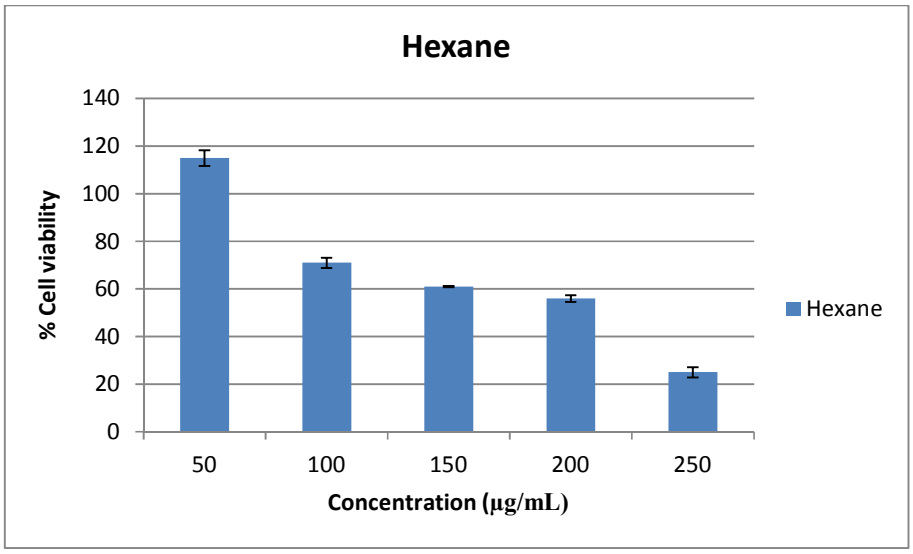

Fig. 1. Activities of HeLa cells at different concentration (ug/mL) when treated with hexane extract. Each bar represents the mean of triplicate samples. Error bars represent the standard deviation. A probability value of p< 0.05 was considered significantly

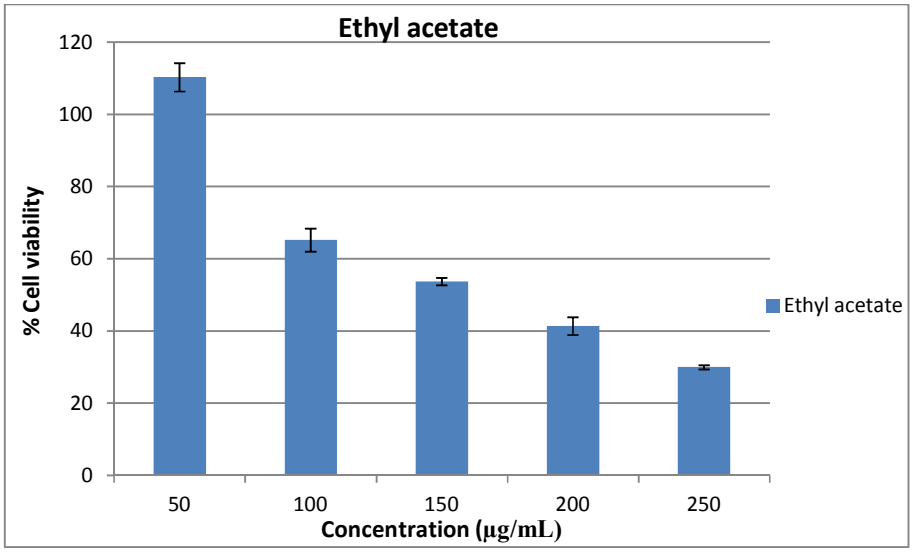

Fig. 2. Activities of HeLa cells at different concentration (ug/mL) when treated with ethyl acetate extract. Each bar represents the mean of triplicate samples. Error bars represent the standard deviation. A probability value of p< 0.05 was considered significantly

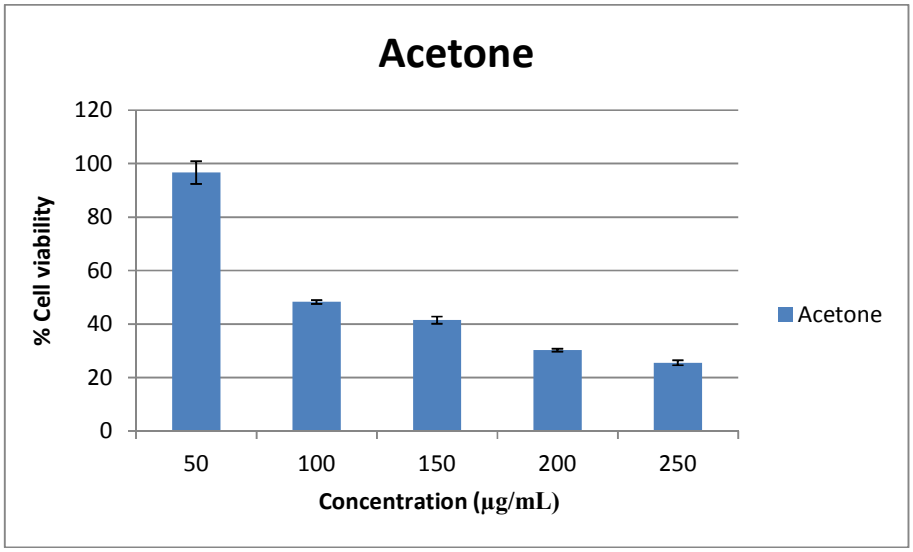

Fig 3. Activities of HeLa cells at different concentration (ug/mL) when treated with hexane extract.. Each bar represents the mean of triplicate samples. Error bars represent the standard deviation. A probability value of p< 0.05 was considered significantly

flavonoids as being a major class of bioactive components in *Anthocleista djalonensis* .These biologically active compounds may be responsible for the *in-vitro* cytotoxic activity of root extract against the HeLa cell lines. The extract cytotoxicity was carried out in comparison to Emetine as positive control. Emetine demonstrated a CC_{50} value of 0.01049 µg/ml.

Table 2. The CC_{50} of extracts against Hela cells

Fraction	Cytotoxicity(CC_{50})
Hexane	241.01231± 3.97201
Ethyl acetate	170.01693 ± 1.93466
Acetone	97.00001 ± 1.25901
Emetine	0.01049 ±0.00001

4. CONCLUSION

This present study reveals the extracts of *A. djalonensis*as a 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.The combination of this new therapy with conventional therapies, may offer high therapeutic efficacy of little or no side effect against cancer.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

Authors thank Professor Rui Krause and his research group at Department of Chemistry, Rhodes University for allowing the use his laboratory.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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