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Research Article

Screening of Selected Nigerian Medicinal Plants/Seeds for Growth Inhibitory Activity against Human Leukemia HL-60 Cells and DPPH Radical Scavenging Activity

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ABSTRACT

Leukemia is the most common cancer in children, accounting for a prominent percentage of cancer-related death in that age-group. It is therefore very important to prevent and treat this cancer. In this study, the anticancer effects of 6 different parts of 4 selected species of medicinal plants in Nigerian, especially those grown in South-Western and South-Eastern regions, were investigated on Human leukemic-HL-60 cell line to identify potential natural alternatives for the development of antileukemic drugs and possible *in vitro* antioxidant activity. The plants / seeds were gathered from selected regions in Nigeria and their aerial parts extracted through repeated maceration in methanol 70%. The cytotoxic activity of extracts on human leukemic- HL-60 cell line was evaluated by Cell Counting Kit (CCK-8) assay 48 hours after treatment. The *in vitro* antioxidant activity was evaluated using DPPH radical scavenging method. *Polyalthia longifolia* seeds and leaves had marked anti-proliferative activity against HL-60 cells showing from the inhibitory concentration at 50% (IC_{50}: 52.1 and 56.69 µg/ml respectively). In a time-dependent fashion, *Polyalthia longifolia* Stem bark showed the lowest DPPH radical survival rate, demonstrating its promising antioxidant status. Our findings show that, Polyalthia longifolia seed possess both growth inhibitory potential against leukemic cell lines and in vitro antioxidant activity, therefore a good candidate for further investigation for active chemopreventive or/and therapeutic principles. Also, a further exploration via mechanistic studies for possible mechanism of cytotoxicity against leukemic and other cell lines is recommended

Keywords: *Leukemia; Phytomedicines; Radical scavenging activity; Antiproliferative, Drug discovery*

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INTRODUCTION

Leukemia is a broad term describing various types of blood cancers originating from the bone marrow, leading to a high number of dysfunctional blood cells (Greim et al., 2014). These cancer cells are largely undeveloped and often referred to as blasts or leukemic cells (Sharma et al., 2018). Leukemia features several symptoms including fever and chills; bleeding and bruising; increased risk of infections; persistent fatigue/weakness; swollen lymph nodes enlarged liver or spleen, etc. (Davis et al., 2014).

Leukemia is the most common type of cancer amongst children and the leading cause of cancer-related death in this age group, accounting for about 28% of all cancers in children. The most common types in children are acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) (www.cancer.gov.) Globally, it is projected that there will be about 60,530 new cases of leukemia, accounting for 3.4% of all cancer cases in 2020; and an estimated 23,100 deaths resulting in 3.8% of all cancer related deaths in 2020 (www.seer.cancer.gov.) . In Nigeria, paucity of data restricts specific epidemiology of this disease; however, the global cancer observatory states that leukemia is among the top 10 cancers and cause of cancer-related deaths (www.gco.iarc.fr.). Standard chemotherapeutic regimens have been employed over the years in the management of all the forms of leukemia in children and adult. However, these regimens present life-threatening adverse effects that could affect overall quality of life (Paul et al., 2020). These unwanted effects include immunosuppression, thrombocytopenia, alopecia, secondary malignancies, nausea and vomiting, etc. (Airley, 2009; Krisl and Doan, 2017). In developing countries, the availability and affordability of these chemotherapeutic agents still pose a major challenge. All of these challenges, though not limited to the management of leukemia could impede on the overall management of cancers (Sullivan et al., 2015). These

epidemiological projections present a dire need for alternative less toxic, effective and affordable therapies that will serve as chemopreventive or therapeutic agents used as adjuvant to counteract this growing morbidity and mortality trend.

In this wise, it is expedient to discover cytotoxic plants against various cancers, with a particular focus on leukemia, which, coupled with their lower side effects, can serve as adjuvant and chemopreventive replacement to chemotherapy and other life-threatening treatment regimens (Kooti et al., 2017; Afkhami-Ardakani et al., 2017; Wang et al., 2019). These plants are rich in several phytomedicines with proven medicinal uses (Ghasemi & Lorigooini, 2016; Heidari-Soreshjani et al., 2017). Nigeria, especially South-West and South eastern regions are rich in several food plants with marked medicinal properties. The climatic conditions of these areas have enriched these selected plants with high concentrations of phytochemicals with various therapeutic effects

Taken together, our aim was to investigate certain medicinal plants in Nigeria, especially those growing in South-Western and South-Eastern region of Nigeria, whose anti-leukemic potentials have not been verified via inhibitory activity against leukemic cell lines, coupled with their *in vitro* antioxidant activity. Some of these plants have been used as anti-inflammatory; immune-boosting and analgesics according to folkloric findings. However, scientific screening is essential as a "kick-off" point to verifying these claims and eventually be used to produce more efficient and novel phytotherapies to treat leukemia and other oxidative-stress linked pathologies.

MATERIALS AND METHODS

Plant material: The plants and seeds: *Polyalthia longifolia* (PL)- leaves, seeds and stem bark; *Vernonia amygdalina* (VA)- leaves; *Gongronema latifolium* (GL)- leaves; *Tetracarpidium conophorum* (TC)- seeds (Plate 1), were collected locally in different parts of South-Western and South- Eastern Nigeria. The various plants were authenticated by a botanist of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A specimen voucher number was assigned to each plant and deposited in the herbarium.

Preparation of Extracts: The plant/seed enlisted were cleaned, air-dried, pulverized to powder in a mechanical grinder and macerated in methanol (70%) at room temperature for a period of seven days and then filtered. The residues were further reconstituted in methanol for another seven days to get as much yield as possible for quantitative and qualitative assays. Both filtrates (7th and 14th day) were concentrated using a rotary evaporator at temperature of less than 35^{O}C. The concentrates were left to air dry in order to evaporate the remaining methanol present, the percentage yield of the extract was calculated (Adam et al., 2019). Samples were dissolved in DMSO % 0.1(dimethylsulfoxide). Finally, the extracts were diluted in RPMI 1640 to a final concentration of 5mg/ml.

Reagents and Assay kits: RPMI 1640 and DMSO were obtained from Wako Pure Chemical industries, Ltd. Heat Inactivated (HI) bovine serum and phosphate buffer solution (PBS) was obtained from GIBCO (New Zealand. Cell Counting Kit-8 (CCK-8) for cell proliferation and cytotoxicity assays was obtained from Dojindo Molecular Technologies Inc (Kumamoto, Japan). UV-visible absorption was measured using Emax precision microplate reader (490 nm).

Cell line and Culture medium: Human leukemia cell line (HL-60) was provided by DS Pharma Biomedical Co., Ltd, (Japan) and cultured in RPMI 1640 media (with L-glutamine and phenol red) supplemented with 10% HI bovine serum, 1% antibiotics, penicillin-streptomycin and kept in the incubator for 4 days at 37℃ in 5% CO2. Cell viability was measured using the Cell Counting Kit- 8 (CCK-8) assay method.

Cell Counting Kit-8 (CCK- 8) assay: Methods described by Afolabi et al. (2017). The HL-60 cells (2.0×104 cells/mL) were seeded in 96-well plates. After 24 h, sample solutions (0 - 100µg/ml) of all the plants/seed extracts were added. Following 48-h incubation, CCK-8 solution (10 µL) was added, and the plates were incubated for an additional 4 h. Visible absorption (490 nm) was measured using a microplate reader. The percentage Cell Viability was calculated with the formula below:

$$\text{Cell Viability (\%)} : \quad \frac{\text{Absorbance of sample well - Absorbance of blank well}}{\text{Absorbance of control well - Absorbance of blank well}} \times 100\%$$

Plate 1
(A) *Polyalthia longifolia* (Leaves & Stem bark); (B) Polyalthia *longifolia* (Seeds); (C) *Vernonia amygdalina* (Leaves); (D) *Gongronema latifolium* (Leaves); (E) *Tetracarpidium conophorum* (Nuts)

DPPH ((2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity: According to the methods described by Kato et al. (2016). A 10 µL amount of sample solutions (1mg/ml) and 190 µL of DPPH solution (78 µM in distilled H_2O/MeOH = 5/3) were added to 96-well plates. The solutions were vigorously mixed and allowed to stand. Visible absorption (λ = 545 nm) was measured after 15, 30, and 60 min using a microplate reader (Emax precision microplate reader, Molecular Devices Japan, Tokyo, Japan). Wells without the compounds were considered as negative controls. At least three replicates were performed for each sample. The percentage DPPH survival rate was calculated with the formula below:

$$\text{DPPH Survival Rate (\%)} = 100 - \frac{\text{Absorbance of sample well - Absorbance of blank well}}{\text{Absorbance of control well - Absorbance of blank well}} \times 100\%$$

Statistical analysis: The dose-response curves of the plants were fitted by means of the computer program GraphPad Prism 6.0 (GraphPad Software, USA), and IC_{50} was defined by regression analysis.

RESULTS

In this study, 6 different plant parts of 4 indigenous plants of Nigerian medicinal plants were investigated. The percentage yield calculation showed that *Tetracarpidium conophorum* (seed) had the highest yield of 34.65% (Table 1). Cell viability studies showed a concentration dependent increase in the cytotoxicity of the extracts against Human Leukemic (HL-60) cell line. Figure 1 and 2 gives the corresponding inhibitory concentration at 50% (IC_{50}) of all the plant/seed assayed for. *Polyalthia longifolia* (leaves) had the lowest IC_{50} at 48 hrs., showing its marked antiproliferative potential, other IC_{50} are recorded.

A test of the radical (DPPH) scavenging potency of the various samples at timepoints of 0, 15, 30 and 60 minutes showed a time dependent decrease in the percentage DPPH survival rate. Polyalthia longifolia (stem bark) showed the least radical survival on a time-dependent fashion (Figure 3).

Table 1:
Screened Nigerian Medicinal Plants/ Seeds with their respective % Extract yield

S/N	Scientific name	Local names	Extract yield (%)
1	*Polyalthia longifolia* (Leaves)	Mast tree	**14.05**
2	*Polyalthia longifolia (Stem bark)*	Mast tree	**15.18**
3	*Polyalthia longifolia* (Seed)	Mast tree	**7.83**
4	*Vernonia amygdalina* (Leaves)	Bitter leaf, "Ewuro"	**9.20**
5	*Gongronema latifolium* (Leaves)	"Utazi"	**9.87**
6	*Tetracarpidium conophorum* (Nuts)	African walnut "Asala"	**34.65**

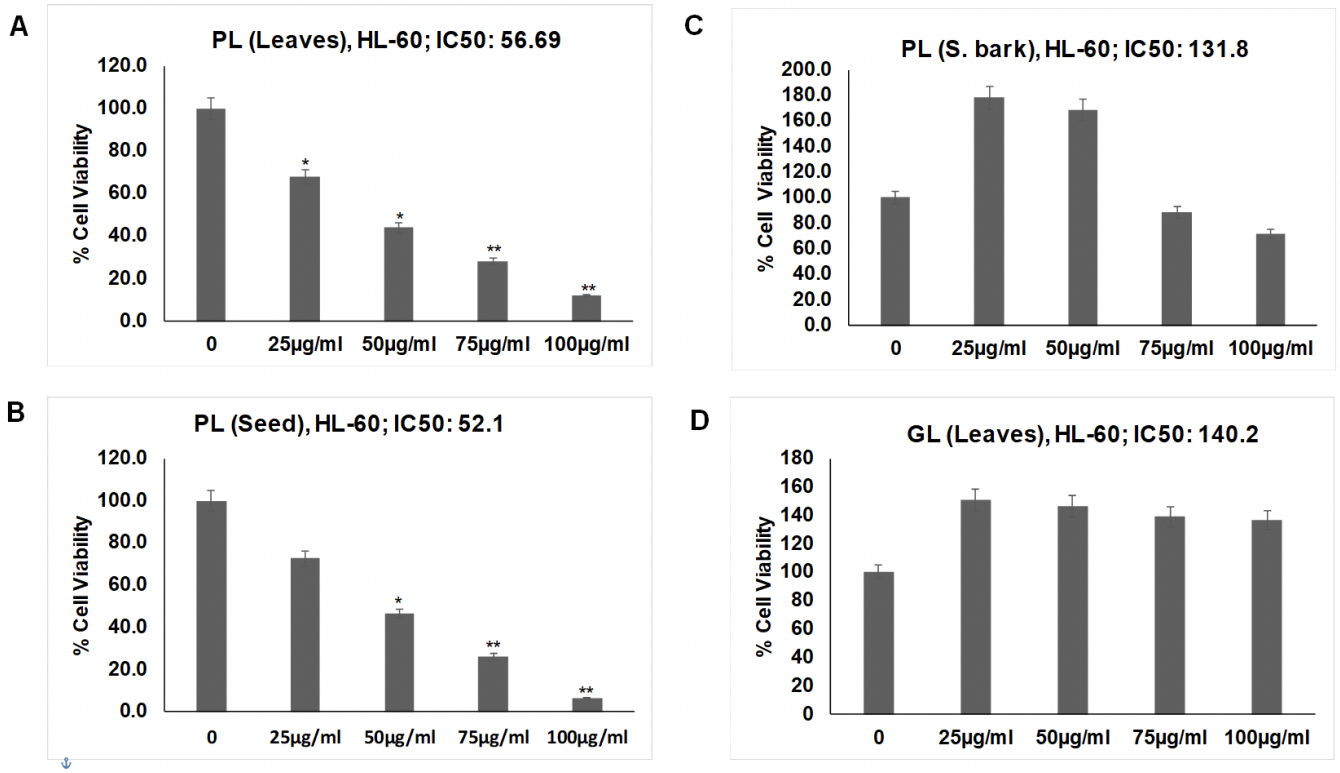

Figure 1
Cell viability assay showing IC_{50} extrapolated for all the samples against Human Leukemia (HL-60) cells at 48 hrs. timepoint. A). The assay showed an IC_{50} of 56.69 for PL (leaves); B) IC_{50} of 131.8 for PL (stem bark); C) IC_{50} of 52.1 for PL (seed); D) IC_{50} of 140.2 for GL (leaves).

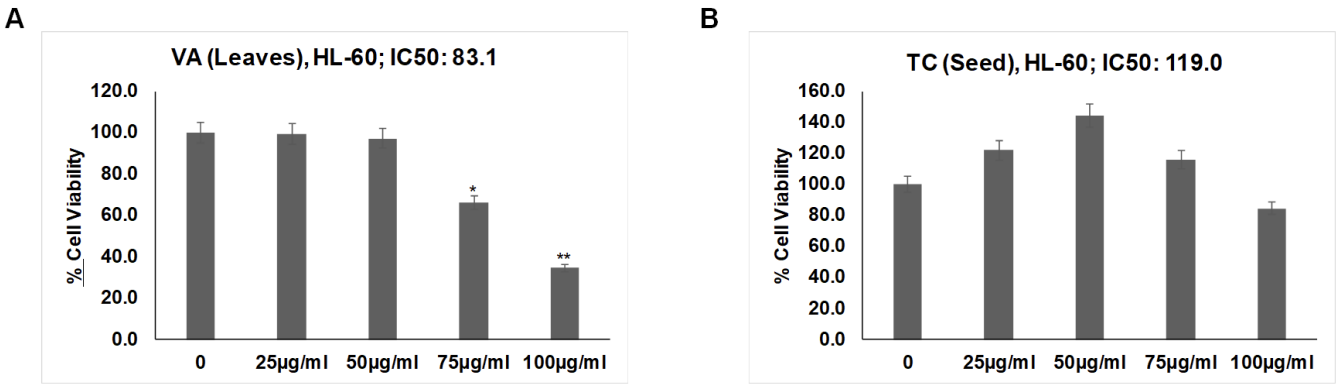

Figure 2.
Cell viability assay showing IC_{50} extrapolated for all the samples against Human Leukemia (HL-60) cells at 48 hrs. timepoint. A). The assay showed an IC_{50} of 83.1 for VA (leaves); B) IC_{50} of 119.0 for TC (seed).

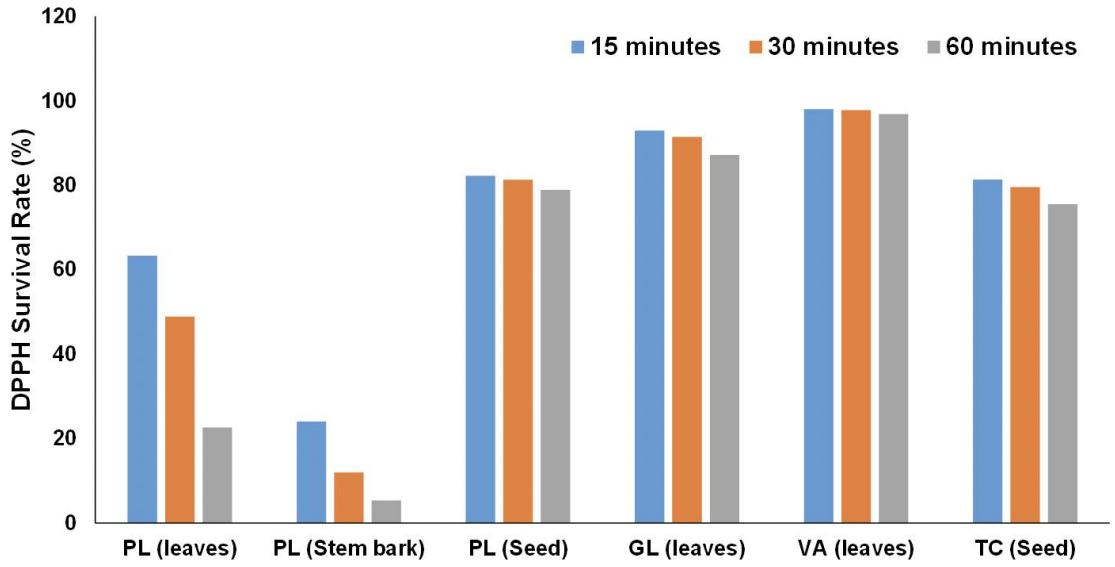

Figure 3
DPPH scavenging activity of the extracts at a final concentration of 1mg/ml (means ± SEMs, n = 2)

DISCUSSION

Taking a clue from the results of several studies conducted on cancer research and the report of IARC of the WHO in 2018 (Wiseman, 2019), in spite of extensive studies on the discovery of novel anticancer drugs, the incidence and prevalence of several cancers remain globally worrisome and in various cases, drug resistance leads to lack of appropriate therapeutic response (Bukowski et al., 2020); therefore, it has become expedient to carry out preliminary screening of natural, affordable and accessible plant based therapies with more potent efficacy that can serve as alternatives/ adjuvant therapies to cytotoxic drugs in a bid to reduce the side effects of current medications and therapies (Schirrmacher, 2019).
Phytotherapeutic approaches have been used as monotherapies or as adjuvant therapies to treat various cancers in recent years (Efferth et al., 2017; Petkova et al., 2019). In this regard, the present study was carried out to screen various parts of 4 Nigerian medicinal plant/seeds for their antileukemic potentials against human leukemic HL-60 cell lines; and DPPH radical scavenging properties. Of the studied plants/seeds, *Polyalthia longifolia* seeds and leaves exerted relatively higher anticancer effects on the HL-60 cells, with the seed showing a greater efficacy as adjudged by the lower inhibitory concentration against the proliferation of the cancer cells. The extracts and pure isolates from the leaves of *Polyalthia longifolia* have been reported to show a great deal of efficacy against various strains of prostate cancer cells (PC3, DU145 and C4-2) (Afolabi et al., 2019), the seeds however are yet to be explored as this study is giving an inkling into its possible greater efficacy over the leaves. Vijayarathna et al. (2017) explored the effect of *Polyalthia longifolia* methanolic extract against human cervical cancer-HELA cell lines, their findings show that the extract induced apoptosis, cell cycle arrest and mitochondrial potential depolarization by modulating the redox status of HeLa cells. This study also showed the moderate cytotoxicity of *Vernonia amygdalina* against the HL-60 cells. *Vernonia amygdalina* has been reported to show marked efficacy *in vitro* against breast cancer- MCF-7 cells (Yedjou et al., 2013), more recent

mechanistic studies on *Vernonia amygdalina* against breast cancer-4T1 cells showed the induction of early and late apoptosis, arrest of the cell cycle progression on the G_2/M phase, and inhibition of PI3K and mTOR expression (Hasibuan et al., 2020). Further exploration of the mechanistic pathway of *Vernonia amygdalina* could provide leads for a novel therapeutic target. So far, the preventive and anticancer effects of many medicinal plants, as well as the effects of their derivatives on different cell lines, are being studied.

Free radical such as DPPH scavenging and general antioxidant prowess have been adjudged essential for proper and robust wellbeing (Okoh et al., 2014). Several disease conditions have been linked to oxidative stress; cancer is not left out of this myriad of pathological conditions. Others include both infectious and non-infectious diseases; neurological pathologies including neurodegenerative disorders (Rekatsina et al., 2020); endocrine disorders such as type 2 diabetes mellitus and several cardiovascular diseases (Liguori et al., 2018). The role of oxidative stress in carcinogenesis has been well elucidated, carcinogenesis follows a multistep process involving both mutation and increased cell proliferation. Oxidative stress can occur through overproduction of reactive oxygen and nitrogen species and have been linked to cancer development (Henkler et al., 2010). The findings from the radical scavenging ability of the selected plant/seeds showed that, on a time-dependent fashion, *Polyalthia longifolia* stem bark showed the most significant reduction in the DPPH radical survival rate, closely followed by the leaves. Not so much has been done to explore the therapeutic benefits of the *Polyalthia longifolia* stem bark against several pathological condition. Since Oxidative stress has been linked to couple of disease condition, a scientific look in this direction might provide new therapeutic leads (Henkler et al., 2010).

In conclusion, according to our results, *Polyalthia longifolia* leaves and seeds have marked anti-proliferative activity against human leukemic- HL-60 cancer cell lines, *Vernonia amygdalina* also showed moderate growth inhibitory potentials against this particular leukemic cell line. However, further studies are required to delineate the possible mechanism by which these particular plants/seeds carry out their cytotoxicity with a major focus on *Polyalthia longifolia* seeds. The radical scavenging prowess of Polyalthia longifolia stem bark potentiates it as a potent antioxidant, further studies into its medicinal efficacy are required to leverage on the benefits of this plant part.

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