The African Journal of Plant Science and Biotechnology ©2012 Global Science Books

Anti-Proliferative Effects of Plant Extracts from Zimbabwean Medicinal Plants against Human Leukaemia Cell Lines

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ABSTRACT

A selected group of 14 medicinal plants was screened for antiproliferative activity against two human leukaemia cell lines Jurkat T and Wil 2 *in vitro*. The Trypan Blue assay was used to assess antiproliferative and cytotoxic activity. The five most potent medicinal plants showed the following order of potency against Wil 2 cell line: *Parinari curatellifolia* > *Aloe barbadensis* > *Croton gratissimus* > *Syzigium guineense* > *Vernonia adoensis* with IG_{50}s of 93, 115, 148, 149.8 and 130 µg/ml, respectively. The plants had comparable proliferation inhibition to the cancer drug doxorubicin. From these species, high levels of cytotoxicity were detected in extracts from *Parinari curatellifolia* and *Aloe barbadensis* to Wil 2 cell line at concentrations of 500 and 1 000 µg/ml. *Croton gratissimus*, *Syzigium guineense* and *Vernonia adoensis* extracts were found to be antiproliferative and not cytotoxic at the same concentrations. *P. curatellifolia* extract at a concentration of 10 µg/ml reduced cell proliferation of Jurkat T cells by 70% after 48 h of incubation. Studies were also carried out where the extract from *P. curatellifolia* combined with doxorubicin at concentrations 10 to 0.50 µg/ml were tested for antiproliferative activity against a Jurkat T cell line. The top five plant extracts had IG_{50}s of less than 150 µg/ml. The results show that plants used traditionally for treatment of diseases such as tuberculosis, mycosis and diarrhea can be used to inhibit cell proliferation in leukaemia cell lines. The extract from *P. curatellifolia* could be a potential source of lead compounds that may be used as anticancer drugs. The plant extracts that showed cytotoxicity and inhibition of cell growth will be further characterized to identify the active fractions and compounds.

Keywords: anticancer, Jurkat T cells, *Parinari curetellifolia*, proliferation, Wil 2 cells, Trypan blue
Abbreviations: ATCC, American type culture collection; **DMSO**, dimethyl sulphoxide **FBS**, foetal bovine serum; $\mathbf{IG_{50}}$, growth inhibition that bring about 50% reduction in the number of cells **RPMI**, Roswell Park Memorial Institute

INTRODUCTION

Leukaemia is a cancer of the blood-forming organs where white blood cells are created in very high numbers at the expense of other blood cells (Ozawa *et al*. 1984; Yan *et al*. 2011). The blood eventually becomes constituted of inadequate levels of cells such as red blood cells and platelets (Szczylik *et al*. 1991). The body will have no ability to transport oxygen and nutrients around the body. Acute leukaemia is when the cancer causes the blood forming organs to produce white blood cells that are not fully differentiated and is fatal unless treatment is commenced upon diagnosis because the patient lacks functional white blood cells (Ross *et al*. 1994). This type of leukaemia occurs in children, especially infants. Chronic leukaemia mostly occurs in adults and in this case the blood forming organs retain their ability to over-produce fully differentiated cells despite the cancer (Ross *et al*. 1994). Leukaemia risk is reported to be increased for subjects with greater than 16 parts per million (ppm) cumulative exposure a year or with greater than 8 ppm intensity of benzene as shown by (Infante *et al*. 2006). There have been 415 reported cases of leukaemia recorded in Zimbabwe from a population of 12 million people. Zimbabwe has the 4th highest number of leukaemia cases in the southern Africa region (Chokunonga *et al*. 2005).

The current treatment of leukaemia is anticancer drug-based chemotherapy, biological therapy and bone marrow stem cell transplants (Liu *et al*. 2004). Chemotherapy uses one or more drugs to destroy cancer cells, and is often accompanied by the development of drug resistance and severe side effects (Yan *et al*. 2011). Therefore, it becomes imperative to develop other potential therapeutic agents for the treatment of the disease. One approach to discover novel lead compounds against cancer is the consideration of ancient ethno-medicinal knowledge and the investigation and screening of locally available natural resources (Ozmen *et al*. 2010). Doxorubicin, vinblastine, vincristine and *cis*-diamminedichloroplatinum(II) (CDDP) represent some of the current standard chemotherapeutic drugs used in the treatment of solid and blood cancers (Glass *et al*. 2003). The drugs vinblastine and vincristine have been isolated from *Catharanthus roseus* (rose periwinkle), a wild plant native to Madagascar (Gaines 2004). The identification of plant compounds with anticancer properties is vital for cancer research. Although there are many therapeutic strategies to treat leukaemia, high systemic toxicity and drug resistance limit successful outcomes in most cases (Liu *et al*. 2004). Accordingly, searches have intensified to find novel compounds for leukaemia drug development. In drug discovery or drug assessment using cell lines, researchers aim to find compounds that lead to the triggering of apoptosis in diseased cells such as cancer or HIV infected cells (Klos *et al*. 2009). A candidate drug is, therefore, introduced to the cells and its effects ascertained. The most favourable is a compound that is potent at low concentrations and discriminates between diseased and normal cells (Cochrane *et al*. 2008).

The combination of phytochemicals with chemotherapeutic drugs which enhance drug efficacy while reducing toxicity to normal tissues could be one such approach to cancer treatment (Li *et al*. 2009). In addition, it has also been suggested that the combined effect of natural products may improve the treatment of proliferating cancer cells (Cheah *et al*. 2009). Several herbs and plants with diver-

Received: 27 January, 2011. Accepted: 26 November, 2011.

Original Research Paper

Table 1 Plants that were used in this study, their ethnobotanical uses in Zimbabwe and other countries.

Family	Plant name and Authority	Vernacular name	Voucher number	Traditional medicinal uses of plants
Fabaceae	*Cajanus cajan* (Druce)		N8E7	Stomach ailments (Iwawela *et al.* 2007).
Myrtaceae	*Callistemon citrinus* (Curtis Skeels)		UZ2E7	Hemorrhoid treatment (Oyedeji *et al.* 2009).
Combretaceae	*Terminalia pruniodes* (Lawson)	*Mudziyashe*	N6E7	Diarrhea (Ruffo *et al.* 1991).
Asphodelaceae	*Aloe barbadensis* (Mill.)	*Gavakava*	N11E7	Sap is used to treat skin rushes and the leaves are prepared and used to treat tuberculosis (Chokunonga *et al.* 2004).
Combretaceae	*Combretum apiculatum* (L.)	*Muruka*	C9E7	Coughs,diarrhea,snake bites stomach ache (Ruffo *et al.* 1991).
Araliaceae	*Cussonia natalensis* (Sond)	*Mutobvi*	UZ9E7	Diarrhea (Ruffo *et al.* 1991).
Euphorbiaceae	*Croton gratissimus* (Burch)	*Gunukira*	UZ13E7	Malaria, rabies, gonorrhea, wounds, Ascariasis, internal worms (Iwawela *et al.* 2007).
Euphorbiaceae	*Euphorbia tiraculli* (L.)		N10E7	Removal of benign moles using the latex (Iwawela *et al.* 2007). Rabies treatment.
Chrysobalanaceae	*Parinari curatellifolia* (Planch ex Benth)	*Muhacha*	C6E7	Facilitates conception in women (Chigora *et al.* 2007).
Myrtaceae	*Syzigium guineense* (Will D.C)	*Mukute*	C12E7	Tuberculosis, fevers (Chigora *et al.* 2007).
Anacardiaceae	*Rhus lancea* (Barkely)	*Muchokochiana*	C11E7	Stomach ailments, fevers (Chokunonga *et al.* 2004).
Fabaceae	*Xeroderris stuhlmanni* (Mend)	*Murumanyama*	C4E7	Stomach ailments (Iwawela *et al.* 2007).
Asteraceae	*Vernonia adoensis* (Bip ex Walp)	*Musikavakadzi*	C1E7	Induce birth or carry out abortions (Iwawela *et al.* 2007).

sified pharmacological properties are known to be rich sources of chemical constituents that may have potential for the prevention and/or treatment of several human cancers (Mesquita *et al.* 2009). Humans have used plant products for centuries as spices and flavourings with no observed side effects (Iwawela *et al.* 2007). In recent years humans have incorporated the use of herbs and plant products in disease treatment (Iwawela *et al.* 2007). This mixture of some herbal medicines with conventional medicines tends to have negative results. Any potential drug must, therefore, be preclinically assessed for its interaction with the conventional medicine of that field (Szliska *et al.* 2008).

Natural plant product extracts have been identified as possible anticancer agents as they exhibit antimutagenic and antiproliferative characteristics (Pinmai *et al.* 2008). Most (70%) of all present antileukaemia drugs have been derived from plant compounds or their derivatives (Pujol *et al.* 2007). In order to find alternative ways to treat cancers, there is a need to evaluate plants with ethno-medicinal history and to examine the mechanisms responsible for the anticancer effects of plant-based drugs (Leong *et al.* 2011). The present study, therefore, was aimed at investigating the effect of 14 Zimbabwean medicinal plants on leukaemia cell lines Wil2 and Jurkat T-cells. This study was carried out in order to identify novel therapeutic compounds for possible leukaemia drug discovery and development.

MATERIALS AND METHODS

Cells and reagents

Two human cell lines were used for antiproliferative screening. ECACC strain Jurkat E6 (T-cell lymphocytic cell line) cells purchased from Sigma Aldrich, Germany and an ATCC strain Wil 2 (B cell lymphocytic cell line) was a kind gift from Professor Mampuru from the University of Limpopo, South Africa. The 14 plants listed in **Table 1** were collected from three provinces in Zimbabwe namely, Mashonaland West, Mashonaland Central and Metropolitan and identified by a taxonomist, Mr Chris Chapano, of the National Botanical Garden of Zimbabwe. Voucher specimens were deposited at the Biochemistry Department at the University of Zimbabwe. Doxorubicin, RPMI 1640 Medium, penicillin streptomycin, dimethyl sulfoxide, methanol and all other chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Cell culture

Cell lines Wil 2 and Jurkat T were cultured in RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS) and 1% penicillin and streptomycin cocktail. Cells were inspected and counted using a haemocytometer daily for growth assessment and contamination checks. Cells were incubated in a 2406 Shel Lab carbon dioxide incubator (Sheldon Mfg., Cornellius, USA) with 5% carbon dioxide at 37°C. The trypan blue dye exclusion assay was used to monitor cell viability. Cells that absorbed the dye and appeared blue under an inverted microscope were considered dead (Masters 2000).

Preparation of methanolic extracts

The samples were separated according to the part of the plant such as leaves, roots stem and bark. Samples were separately dried in an oven at 50°C and ground to a fine powder using a two-step electric blender (Cole Parmer Instrument Co., Vernon Hills, USA). The ground powder was placed in a 200-ml flask containing 100 ml of methanol. The flask was left shaking overnight in an incubator at 100 rpm for maximum extraction to occur (Cochrane *et al.* 2008). The sample was then filtered through Whatman 2® paper (Sigma Aldrich, Taufkirchen, Germany). The filtrate was fan dried until only sludge remained. The sludge was weighed and then redissolved in 10 ml of DMSO and the concentration noted. The extract was then stored at -20°C until required.

Antiproliferative and cytotoxic assays *in vitro*

Wil2 and Jurkat T cells were seeded in 6 well COSTAR® culture plates, Corning, St Louis, USA) at a density of 800,000 cells/well. The methanolic extracts were then dissolved in DMSO to the concentrations of 0, 50, 100, 250, 500 and 1000 µg/ml. Doxorubicin as the positive control was used at 10 µg/ml. The various concentrations were added to the culture wells in triplicate. Samples were collected at 1, 3 and 6 h and viable cells counted using the trypan blue dye exclusion assay. Plant extract effectiveness was assessed by the IG_{50} to determine the concentration for 50% growth inhibition. These are interpolated values from the dose-response graphs. Preliminary anticancer screening results showed that the extract from *Parinari curatellifolia* had the highest antiproliferative activity at a concentration of 50 µg/ml. Further work was then carried out to elucidate the lowest concentration of this extract that could be potent against the leukaemia cell lines. Methanolic extracts from *P. curatellifolia* were dissolved in DMSO into test concentrations ranging from 0.50 to 10 µg/ml. The different concentrations of extracts from *P. curatellifolia* were screened for their ability to inhibit Jurkat T and Wil2 cell growth as described above. The positive control was doxorubicin used at concentrations of 0, 0.50, 1.25 and 10 µg/ml. The Trypan blue dye exclusion assay was used for counting viable cells (Naranyanan *et al.* 2005). The response parameter IG_{50} was calculated to determine the concentration for 50% growth inhibition of tumor cells.

Drug and plant extract combinations

Once cell population reached 2×10^6 cells/ml, cells were seeded in 6-well COSTAR culture plates (COSTAR, St. Louis, USA). The

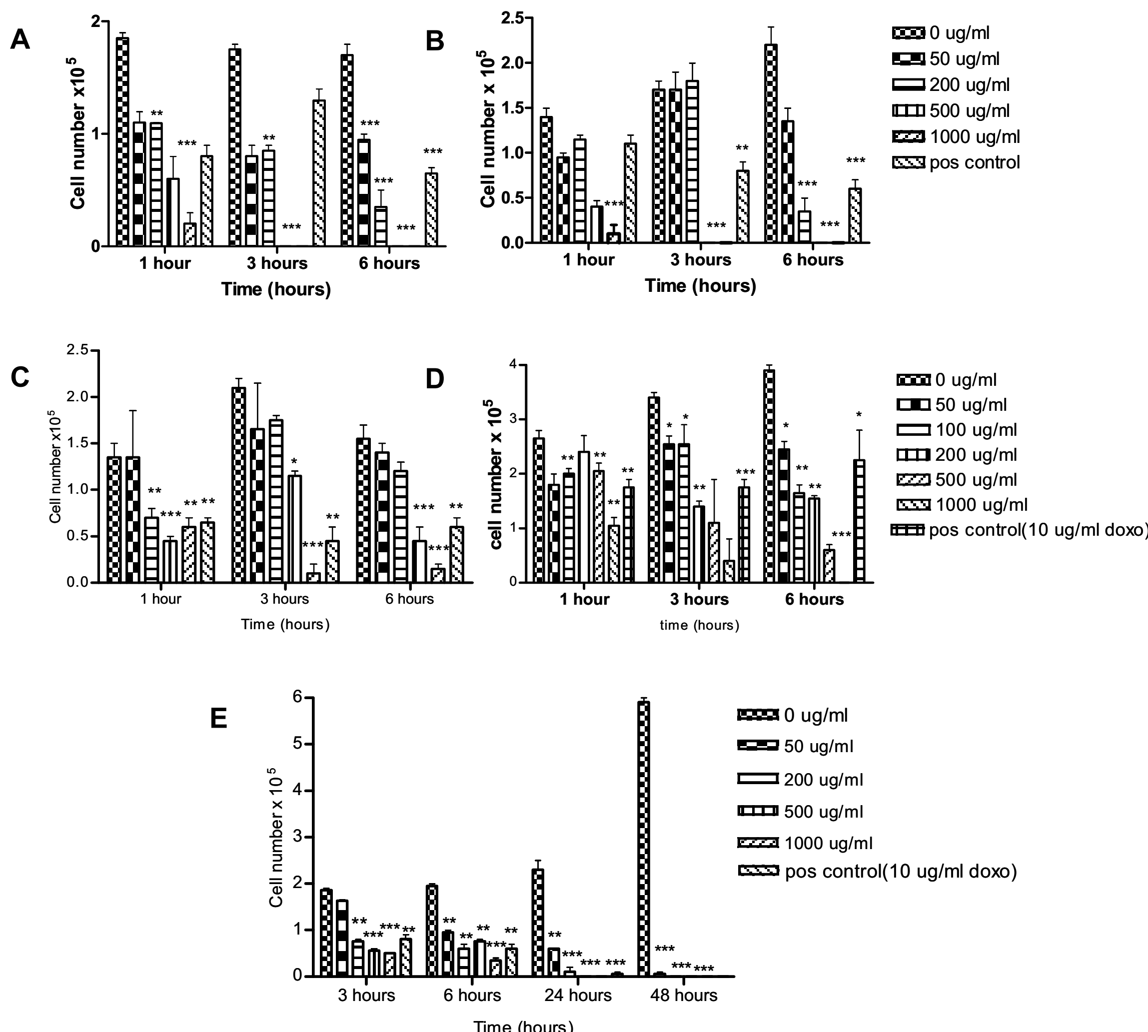


Fig. 1 Wil 2 antiproliferation activity of plant extracts. *Parinari curatellifolia* (**A**), *Aloe barbadensis* (**B**), *Syzigium guineense* (**C**), *Croton gratissimus* (**D**) and *Vernonia adoensis* (**E**) on Wil2 cells over a period of 24 h (A, B, C, D and E respectively). Statistics: *t*-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Readings were carried out in triplicate and each point represents the mean ± standard deviation.

drug, doxorubicin and the extract from *P. curatellifolia* were added to each flask at 10, 1.25, 1.00 and 0.50 µg/ml and placed in the incubator for 48 h. The cells were then counted using the Trypan blue dye exclusion assay. The combination index (CI) was then calculated using the formula:

$$CI = \frac{\text{Inhibition of plant extract + doxorubicin}}{\text{Inhibition of doxorubicin}} \times 100\ \%$$

The CI values were then interpreted as follows: < 0.5: synergism; > 0.5 to 1.0: no interaction; and 1.0 to 4.0: antagonism (Zhao *et al.* 2004).

Statistical analysis

Linear regression analyses were used to determine the IG_{50} of the plant extracts, the concentration that inhibits cell proliferation by half. Numerical data for treatment of cells were analysed using the Student's *t*-test using Graphpad™ version 4 for Windows (Graphpad™ Software Inc., San Diego, California, USA). *P* values of 0.05 or less were reported as significant.

RESULTS

Antiproliferative activity *in vitro*

Of the 14 plant extracts screened, 5 were found to have the highest proliferation inhibition activity. The criterion for potency was considered as the lowest extract concentration that could inhibit proliferation by at least 50% in the shortest period of time. Upon observing that this criterion was met after 3 h of incubation as shown in **Fig. 1**, the cut-off

Table 2 The effects of combining *Parinari curatellifolia* extract and doxorubicin on Jurkat T cells.

Concentration (µg/ml)	Index	Interpretation
10	0.00	Synergism
1.25	0.60	No interaction
1.00	0.54	No interaction
0.50	0.60	No interaction

Key: values < 0.5 synergism, > 0.5 to 1.0 no interaction and 1.0 to 4.0 antagonism (Zhao *et al.* 2004).

period was then set at 3 h. The 5 extracts where found to potent in the order: *Parinari curatellifolia* > *Aloe barbadensis* > *Croton gratissimus* > *Syzigium guineense* > *Vernonia adoensis* against Wil 2 cells. Extracts from the 5 plants were approximately 1.5-, 2-, 1-, 1- and 1-fold more effective on Wil 2 cells than 10 µg/ml doxorubicin, as shown in **Table 2**. For the extracts from *P. curatellifolia* and *A. barbadensis*, there was an unexpected significant increase in proliferation at concentrations from 50 to 200 µg/ml. *S. guineense*, *C. gratissimus* and *V. adoensis* extracts displayed dose-dependent effects on cell proliferation. The concentrations resulting in 50% growth inhibition (IG_{50}) for these species against the cell line Wil 2 are shown in **Fig. 2**.

The highest inhibition activity ($IG_{50} < 100$ µg/ml) was shown by the extract from *P. curatellifolia* which had a IG_{50} of 93 µg/ml. The extracts from *P. curatellifolia* and *A. barbadensis* reduced cell proliferation by 50% and by 100% at 94 and 500 µg/ml, respectively. Concentrations of 500 and 1000 µg/ml of *P. curatellifolia* and *A. barbadensis* were cytotoxic to Wil 2 cells inhibiting proliferation by 100% as

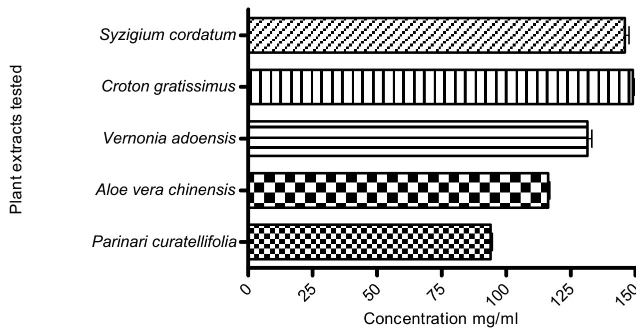


Fig. 2 IG$_{50}$s of extracts from *Parinari curatellifolia, Aloe barbadensis, Syzigium guineense, Croton gratissimus* and *Vernonia adoensis*.

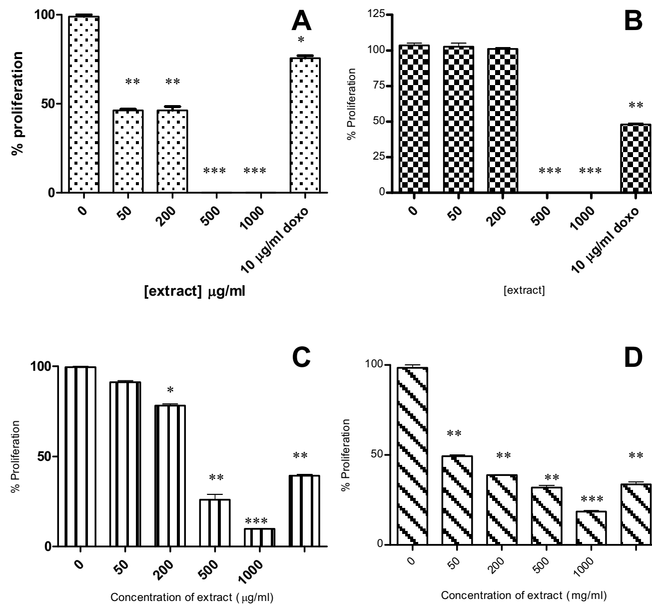


Fig. 3 The antiproliferative and cytotoxic effect of extracts from *Parinari curatellifolia* and *Aloe barbadensis* on Wil2 cells in comparison to anticancer drug doxorubicin (A, B respectively). The antiproliferative effect of *Croton gratissimus*, *Syzigium guineense* and *Vernonia adoensis* on Wil 2 cells (**C**, **D** and **E**, respectively). Statistics: *t*-test,* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Number of readings (n = 3) and each point represents the mean ± standard deviation.

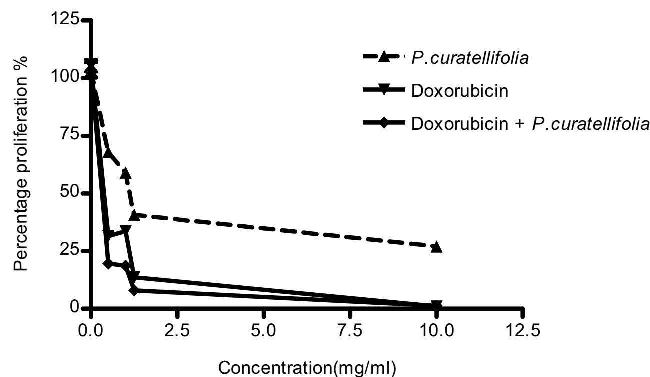
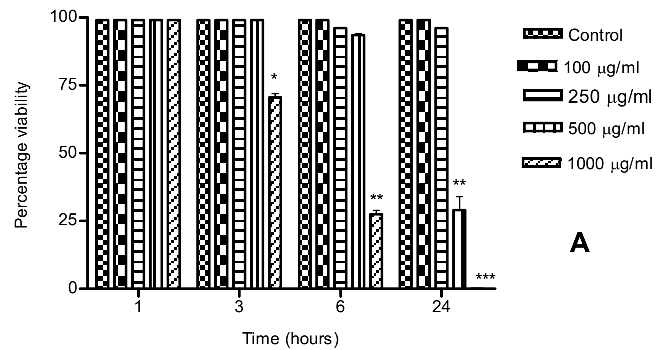
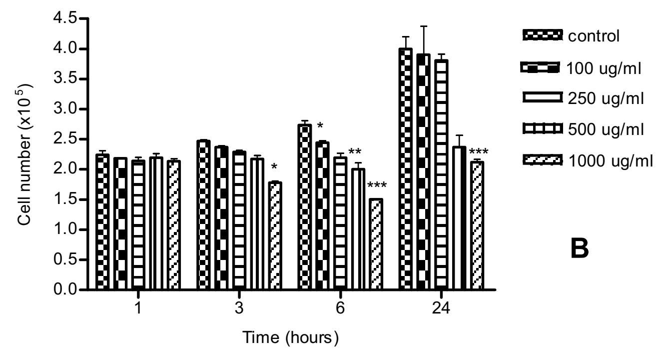


Fig. 4 The effect of fixed concentrations of *Parinari curatellifolia* extract and Doxorubicin on Jurkat T cells after 24 h of incubation. Each reading was taken three times: (n = 3) and each point is the mean ± standard deviation.

shown in **Fig. 3A** and **3B**. The extracts from *C. gratissimus*, *S. guineense* and *V. adoensis* inhibited Wil 2 cell proliferation by 96, 59 and 51% at 1000, 200 and 50 µg/ml, respectively. Extracts from *C. gratissimus*, *S. guineense* and *V. adoensis* exhibited a dose- and time-dependent effect on proliferation of the Wil 2 leukaemia cell lines with IG$_{50}$s of





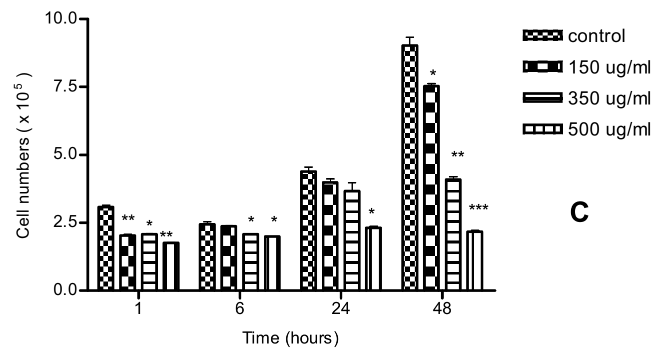


Fig. 5 The effect of plant extract on Jurkat T cell viability and antiproliferative effect of extracts from *Cajanus cajan* and *Euphorbia tiraculli* on A, B and C respectively. Statistics: *t*-test,* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Number of readings (n = 3) and each point represents the mean ± standard deviation.

148, 149 and 130 µg/ml, respectively, as shown in **Fig. 3C-E** and **Table 2**.

The effect of plant extracts was also investigated on Jurkat T cells. Lower concentrations of *P. curatellifolia* extract inhibited proliferation of Jurkat T cells after 24 h of incubation as shown in **Fig. 4**. Concentrations of *P. curatellifolia* extract below 50 µg/ml exhibited potency after longer periods of incubation as compared to the effects seen with the high concentrations as shown in **Fig. 4**. The extract from *C. cajan* was not effective in terms of inhibiting Jurkat T cell proliferation as shown in **Fig. 5A** and **5B**. *C. cajan* extract exhibited significant antiproliferative activity only at a concentration of 1000 µg/ml after 24 h of incubation. *Euphorbia tiraculli* (*E. tirucalli*) extract was antiproliferative against Jurkat T cells in a dose-dependent manner after a short exposure time as shown in **Fig. 5B**. After 24 h exposure time, however, the extract from *E. tirucalli* at a concentration of 50 µg/ml had a significantly higher proliferative effect against Jurkat T cells than preceding concentrations as shown in **Fig. 5B**. Combining the *P. curatellifolia* extract with doxorubicin increased proliferation inhibition in Jurkat T cell as compared to the inhibition observed with

doxorubicin alone as shown in **Fig. 4**. The extract from *P. curatellifolia* was not as effective at inhibiting proliferation at low concentrations of 10, 1.25, 1.00 and 0.50 µg/ml as the effectiveness of doxorubicin at the same concentrations.

In vitro combination assay

Synergism was observed at concentrations of 10 µg/ml of *P. curatellifolia* and doxorubicin while the other concentrations showed no interaction in terms of proliferation inhibition.

DISCUSSION

Ethnobotanical knowledge is an important tool in drug discovery and development (Roberson 2008). For example, without ethnobotany the novel HIV drug prostatin would not have been discovered (Williams *et al.* 2004). The most prominent discovery in terms of cancer treatment was that of the highly effective anticancer drug agents vinblastine and vincristine, that were isolated from *Catharanthus roseus* commonly known as rose periwinkle, a wild tree native to Madagascar (Roberson 2008). Traditional medical practises in Zimbabwe use various plants for the treatment of diseases (Chigora *et al.* 2007). However, there is a general loss of traditional medicinal knowledge due to destruction of plant resources. Since every medicine has a toxic dose, we elected to examine extracts from Zimbabwean medicinal plants, for cancer cell antiproliferative and cytotoxic properties. These plants were selected on the basis that they were shown to have exhibited antifungal properties (Mangoyi and Mukanganyama 2010).

The present study showed that five plant extracts had significant antiproliferative activity against leukaemia cell lines Wil2 and Jurkat T as compared to the drug doxorubicin. *P. curatellifolia* and *A. barbadensis* extracts at 500 and 1000 µg/ml were able to induce death in Wil 2 cells. This could mean that these concentrations were lethal against Wil 2 cells. The extract from *C. cajan* was cytotoxic against Jurkat T cells at 500 and 1000 µg/ml.

The extract from *Parinari curatellifolia* had the lowest IG_{50} of 93 µg/ml against Wil 2 cells after 1 h of incubation. Extracts from *P. curatellifolia* have moderate antiproliferative effects against breast cancer cell lines (Fouche *et al.* 2009). This property could be attributed to the high levels of *ent*-kaurene terpenoids that have been isolated from *P. curatellifolia* leaves (Rundle *et al.* 2001). *P. curatellifolia* Benth root bark collected from Zimbabwe was found to have cytotoxic ent-kaurene diterpenoids 15-oxozoapatlin, 13-methoxy-15-oxozoapatlin and 13 hydroxy-15-oxozoapatlin. These three compounds were cytotoxic against A431 human epidermoid carcinoma cell line at 0.3-0.6 mM (Lee *et al.* 1996). The extract inhibited cell cycle progression in breast cancer cell lines that had defective p53 as shown by Lee *et al.* (1996). There are high levels of terpenoids present in *P. curatellifolia* leaves. The terpenoids could be responsible for inhibiting cancer cell proliferation. This compound was found to show selectivity for leukaemia cell lines (Fouche *et al.* 2009). *P. curatellifolia* extract at 500 and 1000 µg/ml had cytotoxic effects on Wil 2 cells. Although the lower concentrations inhibited proliferation, only the high concentrations of *P. curatellifolia* extract were lethal to Wil 2 cells. Phytochemical constituents in *P. curatellifolia* include alkaloids, flavonoids, digitalis glycosides, terpenes and steroids (Peni *et al.* 2010). The combined effects of these compounds may explain the lethality observed in Wil 2 cells.

A. barbadensis extract at 500 µg/ml was cytotoxic against Wil 2 cells whilst it had an IG_{50} of 115 µg/ml. *A. barbadensis* is traditionally used for easing labour and abortion as it has anti-inflammatory activity (Mukesh *et al.* 2010). In Zimbabwe, extracts from the plant are used to treat skin infections and tuberculosis (Chigora *et al.* 2007). Most compounds that exhibit anti-inflammatory properties have been found in most work to display anticancer activity (Iwawela *et al.* 2007). Aloe emodin is an anthraquinone that has been isolated from *A. barbadensis* (Ni *et al.* 2004). This anthraquinone could be involved in the inhibition of proliferation as these compounds have been reported to bring about cell cycle arrest in cervical cancer cells (Guo *et al.* 2007). Aloe emodin inhibits endothelial cell proliferation but this effect is not cell specific since aloe emodin has also been found to inhibit tumor cell proliferation (Cardenas *et al.* 2006). The cytotoxicity of *A. barbadensis* could be attributed to the anthraquinone aloe-emodin which has been found to induce apoptosis in T24 human bladder cells (Lin *et al.* 2006). Glycoproteins (lectins) and polysaccharides such as acemannan have been found to have anti-cancer effects on *in vitro* models (Reynolds *et al.* 1999). Other active principles of this plant have been shown to inhibit other cancer cell lines such as Ehrlich ascite carci-noma cell (EACC) and in acute myeloid leukemia in the order barbaloin > aloe-emodin > octapeptide > aloesin (Joseph and Raj 2010).

The extract from *V. adoensis* exhibited antiproliferative activity that was comparable to that of the anticancer drug doxorubicin as shown in **Fig. 1E**. *V. adoensis* extract had an IG_{50} of 130 µg/ml against Wil 2 cells. *V. adoensis* is from the family *Asteraceae* whose members are high in sesquiterpene lactones. The high levels of these lactones have been found to correlate with high antiproliferative activity (Fouche *et al.* 2008). This plant has been found to be high in glaucolides, sesquiterpene lactones that are found to relax muscles (Kupcham *et al.* 1969). The glaucolides and sesquiterpene lactones could be the antiproliferative agents of the plant. Glaucolides have been found to display antiproliferative activity against human ovarian cancer cell lines (Khalafalla *et al.* 2009). The sesquiterpene lactone taxol is a well established cancer drug (Kupcham *et al.* 1969). *V. amygdalina* (*Compositae*) is an African medicinal plant well known for producing the anticancer agents vernodaline and vernolide (Kupcham *et al.* 1969). Water and ethanol extracts of *Vernonia amygdalina* killed off the majority (50-75%) of abnormal cells among primary cells harvested from 3 patients with acute lymphoblastic leukaemia (ALL) (Khalafalla *et al.* 2009).

The extract from *C. gratissimus*, also known as *C. Zambesicus*, at 1000 µg/ml had an antiproliferative activity 1.2-fold more than that of doxorubicin (**Fig. 1C**). *C. gratissimus* belongs to the family *Euphorbiaceae* which has been found to have anti-inflammatory and pain-relieving properties (Iwawela *et al.* 2007). The genus *Croton* is well known for its diterpenoid content and different types of diterpenes (phorbol esters, clerodane, labdane, kaurane, trachylobane, pimarane) have been isolated from this genus (Giddy *et al.* 2007). It is possible that the diterpenoid content could be responsible for the antiproliferative activity observed in this study. Diterpenoids have been shown to inhibit cell cycle progression of breast cancer cell lines *in vitro* (Block *et al.* 2002). Few studies are reported in the literature on the antiproliferative effects of *C. gratissimus.* Cembranolides from the stem bark have been shown to have moderate activity against ovarian cancer cell lines (Mulhollad *et al.* 2010). Diterpenes from the dichromethane extract of the leaves were shown to have cytotoxic activity on HeLa cells (Okokon and Nwafor 2010).

S. guineense is traditionally used to treat stomach aches and diarrhea (Tsakala *et al.* 1996; Hamil *et al.* 2000). Ten novel triterpenoids such as betulinic acid, oleanolic acid, asiatic acid, arjunolic acid and hydroxyasiatic acid have been isolated from *Syzigium guineense* (Djoukeng *et al.* 2005). Betulinic acid is one of the triterpenes that have been isolated from *S. guineense* to date; it is a natural product with a range of biological effects and potent antitumor activity. Betulinic acid has been shown to induce apoptotic cell death by triggering the mitochondrial pathway of apoptosis (Fulda *et al.* 2008). The extract from *S. guineense* has the ability to reduce hyperglycaemia, a property that has also been found in the extract of *Catharanthus roseus. C. roseus* is the source of the highly effective antileukaemia drugs, the

vinca alkaloids vincristine and vinblastine (Roberson 2008). Therefore, *S. guineense* might possess similar compounds to *C. roseus* that are responsible for anticancer activity. The high levels of triterpenoids may play a role in the antiproliferative effect observed as triterpenoids have been found to have high anticancer against breast, lung and colon cancer cell lines (Fernandes *et al*. 2000).

Cajanus cajan at 1000 μg/ml was cytotoxic against Jurkat T cells in this study. *C. cajan*, commonly known as pigeon pea, has edible pods that are eaten in tropical areas. An antifungal peptide has been isolated from *C. cajan*; this peptide has an antiproliferative effect toward leukaemia cells (Shirataki *et al*. 2004). Two isoprenylated isoflavone phytoalexins have been isolated from *C. cajan.* Isoflavones have been shown to inhibit proliferation of cancer cells (Dahiya *et al*. 1984). Isoflavones from *Saphora* sp. have shown cytotoxicity and tumor specificity against squamous cell carcinoma and submandibular gland carcinoma (Shirataki *et al*. 2004). The isoflavone, genistein inhibits cell growth in breast and prostate cancer cells *in vivo* and *in vitro* (Sakar *et al*. 2006). Genistein has been found to regulate genes that are critical for the control of cell proliferation, cell cycle, apoptosis, transcription regulation and cell signal pathways (Shirataki *et al*. 2004).

E. tirucalli extract inhibited proliferation of Jurkat T cells in a dose-dependent manner as shown in **Fig. 4**. *E. tirucalli*, commonly known as pencil plant or milk bush, has been found to have ingenol esters which are polyfunctional triterpenoids. The extract has been used traditionally for treatment of warts and cancers in Brazil, Indonesia, Halabar and Madagascar (Cataluna *et al*. 2000). Major constituents are diterpenes from the tigliane (phorbol ester) and from the ingenane (ingenol ester). The plant contains terpenoids such as cycloartanol, euphorcinol and euphorginol (Khaleghian *et al*. 2010). These terpenoids are antileukemic agents (Valadares *et al*. 2006). *E. tirucalli* ethanol extract has been found to modulate myelopoesis and reduce spleen colony formation of tumors (Valadares *et al*. 2006). *E. tirucalli* reduction of tumours may be related to its antitumor activity. Extract from *Euphorbia peplus* tested against cancer cells taken from 8 patients with acute myeloid leukaemia killed 56% and 95% of cancer cells at top and end of the scale (Yadav *et al*. 2002). *Euphorbia peplus* methanolic extract, known as petty surge, has been found to activate protein kinase C which triggers controlled cell death in acute myeloid leukaemia cells (Yadav *et al*. 2002).

The plants whose extracts exhibited the most antiproliferative activity were collected from the Mashonaland West province of Zimbabwe which is 1100 m above sea level. This observation could mean plants collected from different areas could have different compounds present in them. *Callistemon citrinus*, an ornamental plant collected from Australia, Egypt and South Africa have been found to contain different proportions of oils and secondary metabolites. The differences can be attributed to differences in generics and geographic/environmental conditions (Oyedeji *et al*. 2009).

CONCLUSIONS

This study has shown that methanolic plant extracts from *P. curatellifolia* (leaves), *A. barbadensis*, *C. gratissimus*, *V. adoensis* (leaves), *S. guineense* (leaves), *C. cajan* (leaves) and *E. tirucalli* (leaves) have antiproliferative effect against leukaemia cell lines *in vitro*. The results show that the ethnobotanical pre-screening of medicinal plants for drug discovery could yield useful therapeutics and the extract from *P. curatellifolia* could be a potential source of lead compounds that could be used as anticancer drugs. Our data supports the chemotherapeutic potential of medicinal plants from Zimbabwe against leukaemia cell lines *in vitro*. There is need to carry out isolation and identification of the compounds by cytotoxicity-guided fraction and this is work under evaluation. There is need to promote conservation of these plant species and to encourage local farmers in the new resettlement schemes to consider agroforestry.

ACKNOWLEDGEMENTS

The authors would like to thank the International Funding for Science (IFS-F/3413-02) and International Program in the Chemical Sciences (IPICS- ZIM01) for providing with funding for the research. We would like to gratefully acknowledge part-funding from the Academy of Sciences for the Developing World (TWAS) RGA-05-075 RGBIO/AF/AC), Trieste, Italy. The authors would also like to thank Mr. Christopher Chapano, of the National Botanical Garden, Harare, Zimbabwe for the assistance in the taxonomical identification of some plant species.

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