The Effects of Combining Cancer Drugs with Compounds Isolated from *Combretum zeyheri* Sond. and *Combretum platypetalum* Welw. ex M.A. Lawson (Combretaceae) on the Viability of Jurkat T Cells and HL-60 Cells

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Received 13 May 2020; Revised 10 December 2020; Accepted 30 December 2020; Published 18 January 2021

1. Introduction

Noncommunicable diseases are an increasing health problem in both developed and developing countries [1]. WHO estimates that cancer is the leading cause of death from non-communicable diseases, followed by ischemic heart diseases, and lower respiratory tract infections and stroke [2]. Generally, cancer in developed countries is usually a result of lifestyle changes such as obesity, alcohol intake, smoking, and physical inactivity [3]. Despite having a high prevalence of cancers, African countries are not prepared to deal with this burden as they have to fight other diseases such as the human immunodeficiency virus, tuberculosis, and malaria which are also possessing a real health challenge [1, 4, 5]. It is estimated that about 6-7% of new cancer cases are diagnosed every year worldwide [6].
The antioxidant defence mechanism causes cell damage from free radicals (OFR) which is ubiquitous. The OFR-associated lesions that will not result in cell death can stimulate the development of cancer [7, 8]. Endogenous DNA lesions left behind are genotoxic and can induce mutations. Oxygen radicals have been shown to oxidise lipids or proteins, thus generating intermediates that react with DNA forming adducts. Reactive Oxygen Species (ROS) have been observed to facilitate cancer cell proliferation and increase the rate of survival and adaptation to hypoxia [9]. An increase in proliferation of the blood cells in the bone marrow and blood-producing organs leads to a condition called leukaemia [10]. Progression of leukaemia may be sudden (acute) or slow gradual (chronic). Acute and chronic leukaemia mainly occurs in children and adults, respectively. Generally, a malignancy that involves myeloid cells, granulocytes (neutrophils, eosinophils, and basophils), and macrophages leads to myeloid leukaemia, and that which involves T and B lymphocytes is called lymphocytic leukaemia [11].

Drugs used in the management of cancer usually inhibit the growth of tumour cells or kill the tumour cells [12]. There are several classes of anticancer compounds that include: alkylating agents, antimetabolites, cytotoxic antibiotics, plant derivatives, and protein kinase inhibitors [13]. Chemotherapy usually combines several drugs to provide a large cytocidal effect in several tumour cells. Various methods such as dose reduction, cytoprotective agents, alternative drugs, or their analogues such as growth factors are used to decrease the incidences of adverse effects [14].

Modern healthcare practice is also slowly embracing the use of traditional and herbal medicines in the management of various ailments. *Combretum zeyheri* is known by the local community with various names such as large-fruited Bushwillow [15]. The use of *C. zeyheri* has been documented for treating many diseases in both South and Eastern Africa. The plant has primarily been used in the management of hookworm infections, dysentery, coughs, vomiting, sore throat, stomachache, and diarrhoea [16]. *Combretum platypetalum* is a shrub that is less than 30 cm that grows from woody root state and is distributed in Africa in countries such as DRC, Tanzania, Mozambique, Zambia, and Zimbabwe. The shrub has been primarily used in the management of hookworm infections, dysentery, coughs, vomiting, sore throat, stomachache, and diarrhoea [17]. The extract of *C. platypetalum* is cytotoxic to cancer cell lines in vitro [18]. Some compounds from the Combretaceae species have been shown to have anticancer activity [19].

Anticancer drugs used in chemotherapy often fail to treat cancers resulting in unresponsiveness to chemotherapy or recurrence of secondary cancers. For successful cancer treatment, sometimes two drugs are used. The development of drug resistance during chemotherapy decreases the success of chemotherapy [20]. Plants are good sources of compounds that are active as anticancer agents or that can be used as leads to the discovery of new anticancer drugs [21]. This study was set up to investigate the combination of drugs used in the management of cancer with phytochemical constituents from the Combretaceae species. This study provided valuable information about synergistic or antagonistic effects of taking herbal medicines together with conventional drugs. The aim of the study was to investigate the anticancer activity of the compounds isolated from *C. zeyheri* and *C. platypetalum* on Jurkat T cells and HL-60 cells when used singly and when combined with chlorambucil and doxorubicin.

2. Materials and Methods

The chemicals and the cancer cells used in the study were obtained from Sigma-Aldrich Chemicals Company (Munich, Germany). The digital light microscope from Celestron (Los Angeles, USA) and haemocytometer were used to count cells. Incubations were carried out in a CO₂ SHEL LAB incubator (Sheldon Mfg. Inc., Cornelius, USA). Analyses of cell density were done using a Tecan Microplate reader (Tecan Group Ltd., Männedorf, Switzerland). The leaves of *C. zeyheri* and *C. platypetalum* plants were collected in Norton, Mashonaland Central province, Zimbabwe, whose geographical location is 17.8833°S, 30.7000°E, 1364 m above sea level. The plant sample identity was authenticated by Mr. Christopher Chapano, a taxonomist at the National Botanic Gardens (Harare, Zimbabwe). Voucher specimen Nos. N6E7 and N9E7 respectively were deposited at the University of Zimbabwe, Biochemistry Department. Leaves were dried and ground into powder before extraction. A mass of 800 g of powdered *C. platypetalum* leaves or 950 g of powdered *C. zeyheri* was extracted with a DCM:MeOH mixture (1:1 ratio). The DCM:MeOH extract was concentrated under reduced pressure to yield a dark green residue and then submitted to a silica gel (200–300 mesh) column and eluted with mixtures of n-hexane, EtOAc, and MeOH of increasing polarity, giving fractions. *C. platypetalum* was subjected to column chromatography over silica gel 230–400 mesh (Merck), eluted with a gradient of n-hexane (100-0%)/EtOAc mixtures (0-100%) and finally 10% Methanol, and yielded compounds CP 409, CP 404 whilst the *C. zeyheri* extract yielded compounds Cz 453 and Cz 455.

2.1. Identification of Isolated Compounds

The isolated compounds were identified based on their NMR and MS data in corroboration with data from the literature. ¹H NMR spectra were recorded at 400 MHz and ¹³C NMR spectra at 100 MHz. The chemical shifts for ¹H NMR and ¹³C NMR were referenced to TMS via residual solvent signals (¹H, CDCl₃ at 7.26 ppm; ¹³C, CDCl₃ at 77.36 ppm; ¹H, DMSO-d₆ at 2.45 ppm; ¹³C, DMSO-d₆ at 39.43 ppm, ¹H, CD₃OD at 3.31 ppm; ¹³C, CD₃OD at 49.0 ppm). 2D NMR experiments were run using standard pulse sequences. Molecular formulae were determined by electrospray ionization with a 7-T hybrid ion trap and a TOF detector running in positive or negative mode.

2.2. Cytotoxicity Effects of Natural Compounds and Standard Anticancer Drugs on Jurkat T and HL-60 Cells. Compounds used in this study were named as follows: CP 409, CP 404, CZ 453, and Cz 455; these were dissolved in dimethyl sulfoxide and RPMI 1640 media to make final concentrations of 25 μg/ml, 12.5 μg/ml, and 6.5 μg/ml. The two anticancer drugs, chlorambucil and doxorubicin, were dissolved in DMSO and RPMI 1640 to make concentrations of 5 μg/ml,
2 μg/ml, and 1 μg/ml. Cytotoxicity of the natural compounds and anticancer drugs was determined for both Jurkat T and HL-60 cells in 12-well plates using the Trypan blue exclusion assay. After seeding all the reagents, the plates were incubated for 72 hours at 37°C and 5% carbon dioxide in a humidified incubator. The negative control consisted of the cells media only. The positive control chlorambucil was used at 10 μg/ml, and doxorubicin was used at 10 μg/ml. GraphPad Prism 6 was used to determine the GI50 of the compounds exposed to both Jurkat T cells and HL-60 cancer cell lines.

2.3. Cytotoxic Effects of the Combination of the Anticancer Drugs and Phytochemical Constituents. The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was used to measure the effects of combining the anticancer drugs (doxorubicin and chlorambucil) with the phytochemical constituents from C. zeyheri and C. platypetalum. From the GI50 of the compounds and anticancer drugs, the following concentrations were prepared: GI50p 1/2 GI50p 1/4 GI50p 1/8 GI50p 1/16 GI50p and 1/32 GI50p in μg/ml. The compounds were added in equal volumes of 10 μl each. The positive control for the combination assay involving chlorambucil was doxorubicin at 10 μg/ml and that of doxorubicin was chlorambucil at 10 μg/ml. After adding all the reagents, the 96-well microplates were incubated for 72 hours at 37°C in a 5% carbon dioxide humidified SHELL-LAB incubator (Sheldon Mfg. Inc., Cornelius, USA). Absorbance was measured at 590 nm using a Tecan Microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

2.4. Toxicological Effects of Combining Anticancer Drugs and Phytochemical Constituents on Mouse Peritoneal Cells. The induction and harvesting procedure for obtaining viable immune cells from the peritoneal cavity of mice was carried out as described by Ray and Dittel [22]. Toxicological evaluation of the effects of combined compounds was carried out by the combination of CP 404 with chlorambucil. The GI50 concentrations of CP 404 were combined with a GI50 of chlorambucil. RPMI media, compounds (anticancer compounds and phytochemicals), and mouse peritoneal cells were seeded into 12-well plates and incubated for 72 hours. Cell counts were then performed using the Trypan blue exclusion assay.

2.5. Statistical Analyses. Graphical and statistical analyses were carried out using GraphPad Prism version 6. The data were expressed in the form of mean ± standard deviation of the mean. Significant differences between various means of controls and the tests were analyzed using the one-way ANOVA using Dunnett’s Multiple Comparison Post Test with a P value of 0.05.

3. Results and Discussion

Cancer is a debilitating disease that claimed an estimated 9.6 million deaths in 2018 [2]. Drug combinations may be used in the management of cancer as there is a high diversity of tumour malignancy and incomplete use of treatment regimen that encompasses one or more modalities which essentially leads to drug resistance [23, 24]. The mechanism of anticancer drugs is usually different, and a combination of anticancer drugs leads to the cure of cancer or to considerably prolonged life as these combinations present multidrug pathway targets leading to improved therapy [25, 26]. Drug combinations also help in reducing the adverse effects of anticancer drugs, thereby, improving the patient’s quality of life. Anthracyclines such as doxorubicin and biodioxopiperazine such as raxoxane have been combined leading to decreased drug resistance and neoplasm metastasis [24].

3.1. Spectroscopic Identification of Isolated Compounds. Spectroscopic analysis of the pure compounds CZ 453 and CZ 455 revealed that the compounds were waxes. However, CP 404 and 409 were found to be pure compounds. Outlined below is the data for the compound CP 404 (1):

**Compound 1 (CP 404):** myricetin-3-O-glucoside or 3-Oβ-D-glucopyranosyl-3′, 4, 5, 6, 7, 8, 3′, 4′, 5′-pentahydroxyflavone, amber powder; HR-ESI-MS m/z 481.0986 for [M+H]+ (calcd 481.0982) corresponding to C21H20O12.

**Compound 2 (CP 409):** myricitrin or myricitrin-3-O-D-glucopyranoside or 3-Oβ-D-glucopyranosyl-3′, 4, 5, 6, 7, 8, 3′, 4′, 5′-pentahydroxyflavone, yellow powder; HR-ESI-MS m/z 466.1036 for [M+H]+ (calcd 466.1111) corresponding to C21H20O12.

C. zeyheri and C. platypetalum. From the GI50 of the compounds and anticancer drugs, the following concentrations were prepared: GI50p 1/2 GI50p 1/4 GI50p 1/8 GI50p 1/16 GI50p and 1/32 GI50p in μg/ml. The compounds were added in equal volumes of 10 μl each. The positive control for the combination assay involving chlorambucil was doxorubicin at 10 μg/ml and that of doxorubicin was chlorambucil at 10 μg/ml. After adding all the reagents, the 96-well microplates were incubated for 72 hours at 37°C in a 5% carbon dioxide humidified SHELL-LAB incubator (Sheldon Mfg. Inc., Cornelius, USA). Absorbance was measured at 590 nm using a Tecan Microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

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C. zeyheri and C. platypetalum. From the GI50 of the compounds and anticancer drugs, the following concentrations were prepared: GI50p 1/2 GI50p 1/4 GI50p 1/8 GI50p 1/16 GI50p and 1/32 GI50p in μg/ml. The compounds were added in equal volumes of 10 μl each. The positive control for the combination assay involving chlorambucil was doxorubicin at 10 μg/ml and that of doxorubicin was chlorambucil at 10 μg/ml. After adding all the reagents, the 96-well microplates were incubated for 72 hours at 37°C in a 5% carbon dioxide humidified SHELL-LAB incubator (Sheldon Mfg. Inc., Cornelius, USA). Absorbance was measured at 590 nm using a Tecan Microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

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Table 1: Summary of GI<sub>50</sub> of compounds against Jurkat T cells by Trypan blue assay for various concentrations in μg/ml.

| Name of compound | GI<sub>50</sub> (μg/ml) |
|------------------|-------------------------|
| CP 409           | 19.3                    |
| CP 404           | 3.98                    |
| CZ 453           | 6.24                    |
| CZ 455           | 20.3                    |
| Chlorambucil     | 2.99                    |
| Doxorubicin      | 2.50                    |

Compounds isolated from Combretum platypetalum. Compounds isolated from C. platypetalum inhibited the growth of Jurkat T cells with both compounds showing a dose-dependent antiproliferative and cytotoxic inhibition of growth on Jurkat T cells. Of these compounds, CP 404 presented the most potent reduction of cell viability of Jurkat T cells having a GI<sub>50</sub> of 28.7 μg/ml (Table 1). Chlorambucil had GI<sub>50</sub> value of 2.99 μg/ml while that of doxorubicin was 2.50 μg/ml on Jurkat T cells (Table 1). CP 404 showed a similar profile in terms of reduction of cell viability from the lowest concentration of 6.3 μg/ml to the highest concentration of 50 μg/ml (Figure 2). The cell viability was decreased by 64% after treatment with 6.3 μg/ml CP 404 for 72 hours and further reduced by up to 79% with a higher concentration (50 μg/ml) of the compound. Compounds isolated from C. zeyheri also possessed antiproliferative effects and cytotoxicity against Jurkat T cells. The compound CZ 453 had a higher inhibitory effect against Jurkat T cells than CZ 455 though both compounds had dose-dependent antiproliferative activity.

The results summarized in Table 2 showed that all the compounds, as well as standard anticancer drugs incubated with HL-60 cells, exhibited a significant inhibitory effect on the proliferation of these cells. The GI<sub>50</sub> values for chlorambucil and doxorubicin were 1.79 and 3.87 μg/ml on HL-60 cells, respectively (Table 2). The cell viability declined in a concentration-related manner for all the compounds with CP 409 (Figure 3) showing the greatest potency with a GI<sub>50</sub> value of 14.2 μg/ml, though it was not comparable to the positive controls. The flavonoid compound from C. platypetalum, CP 409, had a dose-dependent cytotoxic and antiproliferative effects on HL-60. Compound CP 404, however, possessed the most significant antiproliferative effects at the highest concentration used in this study, and these results are consistent with previous findings [29, 30]. Doxorubicin showed concentration-dependent effects in suppressing the growth of Jurkat T and HL-60 cells in vitro. Comparing with the negative control, that is cells with media only, reduction in the number of viable Jurkat T cells occurred at a concentration of 2 μg/ml (Figure 4). Chlorambucil antiproliferative effects were evaluated in vitro on Jurkat T and HL-60 cells; there was concentration-dependent inhibition of growth of HL-60 cells (Figure 5).

Plants have been a source of bioactive compounds for use as anticancer drugs and source of agents which may be used as chemopreventive agents [31]. Polyphenolic compounds are a part of a large diversity of natural compounds and this group also comprise of flavonoids [32]. Epidemiological studies have revealed that consumption of plants and fruits rich in flavonoids leads to a decrease in the development of a vast array of cancers [33]. According to the National Cancer Institute (NCI), extracts isolated from plants with GI<sub>50</sub> values of ≤30 μg/ml are regarded as active [34]. All the compounds used in the study had a GI<sub>50</sub> value of less than 30 μg/ml and, hence, are regarded as active according to this criterion. High antiproliferative effects of the fraction CZ 453 may be due to the combination of various phytochemicals such as saponins, tannins, flavonoids, and triterpenes acting synergistically against Jurkat T cells and HL-60 cells [32]. Compounds extracted using methanol have been shown to have antiproliferative activity and are cytotoxic against cancer cell lines [35]. Antiproliferative activity is usually different among cancer cell lines; hence, the compounds showed different cytotoxic activity on Jurkat T cells and HL-60 cells as exhibited by
different GI₅₀ values [35, 36, 37]. The flavonoid drug phenoxodiol, which is undergoing clinical trials, has been shown to kill primarily the myeloid, rapidly proliferating cells, and lymphoid blast cells [30]. Decrease in cell viability by Jurkat T cells and HL-60 when treated with compound CP 409 may be attributed to the presence of flavonoids that kill the cells [38]. Flavonoids have been shown to inhibit cell proliferation through cell cycle arrest. A flavonoid baicalein has been shown to cause a decrease in levels of Cdk, cyclin D1, and cyclin B1 cell arrest in human lung carcinoma cells [39]. Methanol plant extracts have been shown to cause cell death by apoptosis and inhibition of G2/M cell cycle arrest [36, 40]. Apoptosis of the flavonoids has been shown to occur as a result of nuclear condensation, DNA fragmentation, and cell shrinkage [36].

3.3. Cytotoxic Effects of the Combination of the Anticancer Drugs and Phytochemical Constituents. The MTT (3-(4, 5-

![Figure 2: Shows the antiproliferative effects of CP 404 on Jurkat T cells after incubation for 72 hours. The percentage of cell viability was compared to cells with cell media only. The results are showing that as the concentration increases, there were statistical significance decreases in cell viability. Compound 1 had a GI₅₀ value of 3.98 μg/ml. The values are mean ± standard deviation (SD) of 4 replicate data sets. Statistical one-way ANOVA (**P < 0.0001).](#)

![Figure 3: The antiproliferative effects of CP 409 on HL-60 cancer cell lines. The graph shows that as the concentration increases, there were statistical significance decreases in cell viability. The percentage of cell viability was compared to cells with cell media only. The values are mean ± standard deviation (SD) of 4 replicate data sets. Statistical one-way ANOVA was used to analyze the results (**P < 0.001).](#)

Table 2: Summary of GI₅₀ of compounds against HL-60 cells by Trypan blue assay for various concentrations in μg/ml.

| Name of compound | GI₅₀ (μg/ml) |
|------------------|-------------|
| CP 409           | 14.2        |
| CP 404           | 28.7        |
| CZ 453           | 16.5        |
| CZ 455           | 29.9        |
| Chlorambucil     | 1.79        |
| Doxorubicin      | 3.87        |

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. The combination index value showed that there was antagonistic effect (CI = 1.0 to 4.0) between CP 404 and chlorambucil as well as doxorubicin. There was, however, death of cells as shown by a decline in cell viability (Figure 7). At higher
concentrations of CP 404, cell death increased; with this, it may be stated that the cell growth of Jurkat T is independent of concentrations of the anticancer drug. In this study, the combination of CP 404 chlorambucil, as well as doxorubicin, exerts an effect that is less than additive showing antagonistic effects in the drug interaction. It has been shown that the activity of a single compound may be reduced when incubated with another [42]. The cell growth of Jurkat T cells is inhibited by treating the cells with CP 404 without chlorambucil or doxorubicin. Previous studies have shown that combined drug action is greater when phytochemicals are added first rather than the converse [43].

Studies have shown that a combination of natural compounds with anticancer drugs increases their cytotoxic effect by various mechanisms such as sensitizing the cells to the drugs, and the effect depends on the dose of the combination [44]. The decrease in cell viability exhibited as a result of incubation with our compounds shows that the phytochemicals in plant fractions might have acted on various targets in the Jurkat T cells [45, 46]. A study was done by Yue et al. [47] that indicated that triterpenes in *Ganoderma lucidum*, a plant used in Traditional Chinese medical practices in the management of cancer, has an enhanced effect inhibiting proteins that cause cell proliferation, cell cycle, and apoptosis when combined with doxorubicin against HeLa cells without necessarily acting in synergy. Cytotoxicity of the compounds may be a result of phytochemicals causing the production of ROS of the anticancer drugs doxorubicin and chlorambucil

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**Figure 4:** The antiproliferative effects of chlorambucil and doxorubicin on Jurkat T cancer cell lines. The graphs show that as the concentration increases, there was a statistically significant decrease in cell viability compared to the cell with cell media only. The values are mean ± standard deviation (SD) of 2 replicate data sets. Statistical one way ANOVA was used to analyze the results (***P < 0.001).

**Figure 5:** The antiproliferative effects of chlorambucil and doxorubicin on HL-60 cancer cell lines. The graphs show that as the concentration increases, there was a statistically significant decrease in cell viability compared to the cell with cell media only. The values are mean ± standard deviation (SD) of 2 replicate data sets. Statistical one way ANOVA was used to analyze the results (***P < 0.001).
Figure 6: The antiproliferative effects of combining CP 404 and chlorambucil on Jurkat T cells. Chlorambucil (0–16 μg/ml) and CP 404 (GI50, 1/2 GI50, 1/4 GI50, 1/8 GI50, 1/16 GI50, and 1/32 GI50) were combined and incubated with Jurkat T cells for 72 hours. The MTT assay was used to determine the number of viable cells present in the sample. When Jurkat T cells were incubated with varying amounts of chlorambucil combined with CP 404, there was no significant difference in the cell viability. Cell density corresponds to cell viability: the higher the cell density, the more viable cells present in the sample. The values are mean ± standard deviation (SD) of 2 replicate data sets.

| Concentration | Cell Density at 590 nm |
|---------------|------------------------|
| 0.000         |                        |
| 0.005         |                        |
| 0.010         |                        |
| 0.015         |                        |

Figure 7: The antiproliferative effects of combining CP 404 and doxorubicin on Jurkat T cells. The antiproliferative effects of CP 404 (GI50, 1/2 GI50, 1/4 GI50, 1/8 GI50, 1/16 GI50, and 1/32 GI50), when combined with doxorubicin (0–16 μg/ml), were determined using the MTT assay to investigate the number of viable cells present in the sample. The results show that at low concentration of chlorambucil, there was no significant reduction in cell density compared to cells with cell (RPMI) media only. The cell density corresponds to the number of viable cells present in the sample. The values are mean ± standard deviation (SD) of 2 replicate data sets.

| Concentration | Cell Density at 590 nm |
|---------------|------------------------|
| 0.000         |                        |
| 0.005         |                        |
| 0.010         |                        |
| 0.015         |                        |
Statistical one-way ANOVA was used to analyze the results mean ± standard deviation of CP 404 and chlorambucil on mouse peritoneal cells. The values [47]. A combination of extracts from *Boswellia serrata* effects of a combination of CP 404 and chlorambucil on mouse peritoneal cells. The Trypan blue exclusion assay was used to determine the number of viable cells present after incubating the combinations for 72 hours. The results show the cytotoxicity effects of a combination of CP 404 and chlorambucil on mouse peritoneal cells. The values are mean ± standard deviation of two separate experiments. Statistical one-way ANOVA was used to analyze the results (*P < 0.05).

3.4. Toxicological Investigations with Mouse Peritoneal Cells.
Toxicological studies are usually done to predict the potential toxicity of the drug during drug development [49]. Toxicological evaluation of the combination of CP 404 and chlorambucil was carried out using mouse peritoneal cells as this combination showed the highest antiproliferative effects on Jurkat T cells. Mouse peritoneal cells or normal human cells are usually used to study the cytotoxic effects of the drugs in *vitro*. Assessment of the cytotoxic assay is usually done by observing morphology, cell viability, differentiation markers, and inhibition of cell proliferation [50]. The capacity of the plant to inhibit growth and decrease cell viability is also an indication of toxicity [51]. Toxicological evaluation of the combination of CP 404 and chlorambucil was evaluated using mouse peritoneal cells as this combination had shown the greatest potency on cancer cell lines. The Trypan blue exclusion assay was used to determine the number of viable cells present in the sample after incubating the combinations for 72 hours.

The results showed that the combination did not lead to a statistically significant reduction in cell viability at the highest concentration of CP 404 and chlorambucil combination used. The decrease in cell viability was, however, minimal with only about 10% difference compared to the controls (Figure 8). In general, the combination did not result in a more than 10% reduction in the number of viable mouse peritoneal cells; hence, it was considered not to be toxic because it has been shown that for a compound to be stated as toxic, it should at least exhibit a 30% decrease in cell viability [52]. Toxicity of anticancer drugs such as doxorubicin is toxic to mouse peritoneal cells by production of Toll-Like Receptor 4 (TLR4) which also causes the cells to be sensitive to endotoxins which may cause the cells to die [53]. Studies have shown that the toxicological effect of phytochemicals is usually minimal to mouse macrophages [54]. Many phytochemicals such as saponins, lectins, starch, polypeptides, and lectins are not toxic to T and B cells [55, 56].

4. Conclusion
The current study was designed to determine the effect of combining compounds isolated from *Combretum zeyheri* and *Combretum platypetalum* against Jurkat T and promyelocytic leukaemia HL-60 cells. The combination of chlorambucil and doxorubicin was the most potent against Jurkat cells exhibiting synergistic effects in a dose-dependent manner. Combination of phytochemicals and standard drugs resulted in a reduction in cell viability that can be attributed to effects of the isolated compounds as the effects of the anticancer drug were notably the same. The most potent combination did not reduce (by more than 10%) the cell viability of mouse peritoneal cells showing that the compounds are not toxic to normal cells. This study contributes to the understanding of the ethnopharmacological use of the plants against cancer in Southern Africa. Plant extracts and phytochemicals from *Combretum zeyheri* and *Combretum platypetalum* can, therefore, be used as lead candidates for anticancer drug development.

Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval
Ethical consideration to carry out the study was obtained from the Joint Parirenyatwa Hospital and College of Health Sciences Research Committee (JREC). The permission to use mice was given by the Animal House Department under the College of Health Sciences, University of Zimbabwe.

Conflicts of Interest
The authors declare that they have no competing interests.
Acknowledgments

The authors acknowledge the assistance of Mr. Christopher Chapano, a taxonomist with the National Herbarium and Botanical Gardens, Harare, Zimbabwe, in the authentication of the plant sample names. Support came from the Swede International Development Cooperation Agency (SIDA), through the International Science Programmes (ISP) (ISP IPICS: ZIM01, Uppsala University, Uppsala, Sweden). ISP IPICS: ZIM01 supported the research under the title “Biomolecular Interactions Analyses.” Support from the Alliance for Global Health and Science (University of California Berkeley) is acknowledged.

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